Molecular mechanisms by which SGLT2 inhibitors can induce insulin sensitivity in diabetic milieu: a mechanistic review

Running Title: SGLT2 inhibitors and insulin sensitivity

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Abstract

Sodium-glucose co-transporter-2 inhibitors (SGLT2i) are a relatively newer class of anti-hyperglycemic medications that reduce blood glucose by inhibition of renal glucose re-uptake, thereby increasing urinary glucose excretion. Although glycosuria is the primary mechanism of action of these agents, there is some evidence suggesting they can reduce insulin resistance and induce peripheral insulin sensitivity. Identifying the molecular mechanisms by which these medications improve glucose homeostasis can help us to develop newer forms of SGLT2i with lesser side effects. We have reviewed the molecular mechanisms and signaling pathways by which SGLT2i therapy improve insulin sensitivity and ameliorates insulin resistance.

Keywords: Sodium-glucose co-transporter-2 inhibitors, Insulin Sensitivity, Insulin Resistance, Diabetes Mellitus, Oxidative Stress, Inflammatory Response.

Introduction

The global prevalence of diabetes mellitus (DM) is rising rapidly [1]. This chronic disorder results in the development of various debilitating complications as well as deaths worldwide [2]. Chronic hyperglycemia seen in DM can induce various pathophysiologic pathways involved in tissue dysfunction such as oxidative stress, inflammation, apoptosis, fibrosis, hyperexpression of growth factors and hemodynamic variations [3]. Various medications have been developed to normalize hyperglycemia and thereby preventing diabetic complications [4, 5].

Sodium-glucose co-transporter-2 inhibitors (SGLT2i) are a newly developed class of agents that reduce hyperglycemia in DM by inhibition of renal glucose re-uptake and thereby increasing the excretion of glucose in the urine [5, 6]. These agents act primarily by inducing glycosuria but there is some evidence that they can also induce insulin sensitivity in peripheral tissues [7, 8]. SGLT2i is also associated with some side effects in patients with DM [9-14]. In this review, we have discussed various molecular pathways involved in the modulation of insulin sensitivity by SGLT2i.

Type 2 diabetes (T2DM) is the most prevalent type of diabetes which account for about 90-95% of patients with diabetes and is linked mainly to inadequate response to insulin (decreased insulin sensitivity) and insulin resistance in the peripheral tissues [15-17]. T2DM is a disorder of glucose homeostasis. Glucose is the most abundant monosaccharide in circulation and the preferred substrate for many types of cells in the human body [18, 19]. This carbohydrate exists mainly as a polysaccharide in starch and then converted by catabolic and enzymatic processes to a hexose monosaccharide [18, 19]. This monosaccharide is a hydrophilic molecule with a molecular weight of 180.156 g/mol, which is too large to pass easily across the cell membrane, thereby needing a specific carrier [19]. Glucose has two major ways for entering into the cells:

(1) via active mechanism by SGLTs and (2) via specific carriers (independent of sodium) [19]. The function of SGLTs is the sections below [20]. Glucose carriers (GLUT) are a family of proteins that provide bidirectional facilitated glucose transport across the cellular plasma membrane [19, 21]. These carriers work without consuming energy and are based on the glucose concentration gradient across the cell membrane. There are at least 14 known GLUT members in human [19, 22]. However, GLUT-1, GLUT-2, GLUT-3 and GLUT-4 are more critical in glucose homeostasis [19]. While GLUT-1 and GLUT-3 are widely expressed, high-affinity and low-capacity transporters, GLUT-2 is high-affinity and low capacity which is expressed in pancreatic beta cells, liver and basolateral side of intestinal and renal tubular cells [19].

Glucose is phosphorylated to glucose-6-phosphate within the beta cells by glucokinase enzyme [23]. This enzyme acts as "glucose sensor" which controls the rate of glucose entering into the islets and in turn controls the rate of insulin secretion [23]. ATP (adenosine triphosphate) synthesis by G6P in beta cells raises the ATP to ADP (adenosine diphosphate) ratio which in turns closes ATP-sensitive K^+ channels (sensitive to sulfonylureas), depolarizes beta cell membrane and increases intracellular calcium concentration by opening the voltage-dependent Ca^{2+} channels in beta cells [23]. Rising the levels of intracellular Ca^{2+} stimulates the exocytosis of secretory granules containing insulin/proinsulin from the beta cells into the circulation [23].

Insulin signal transduction

Insulin signal transduction (IST) or insulin signaling pathway is initiated by insulin binding to the α-subunit of specific receptors known as insulin receptors (IRs), a member of transmembrane tyrosine kinases composed of α and β subunits, which are activated by insulin as well as IGF[1] 1 and 2 [24]. This binding induces structural changes in the β subunit by prompting auto-phosphorylation in tyrosine residues followed by downstream events such as

[1] Insulin-like growth factor

recruitment of different adaptor proteins i.e. insulin receptor substrates (IRSs), Shc[2] protein, and APS protein[3] [19, 25]. These processes provide an appropriate binding site for the IRS-1[4] [25]. Several types of insulin-dependent kinases such as ERK1/2[5], atypical PKC[6], S6K1[7], SIK2[8], AKT, mTOR[9], and ROCK1[10] and other types of kinases including AMPK[11] and GSK3[12] can phosphorylate IRSs to activate them [25, 26]. Activated IRS-1 links to PI3K[13] and activates it and catalyzes the conversion of PIP_2[14] to PIP_3[15] [27]. PIP_3 itself is a potent activator for PKB (protein kinase B also known as Akt), which in turn, facilitates glucose entering into the cells by localization of GLUT-4 and inhibits glycogen synthase kinase leading to more glycogen synthesis [27, 28]. The insulin sensitivity can be modulated in all these steps of insulin signal transduction pathways [29-31].

SGLT2 Inhibitors

Sodium-glucose co-transporters (SGLT) are two distinct forms of active cotransporters (as type 1 and 2) that mainly localized in the brush border of S2 and S3 segments of proximal renal tubules (as well as in intestines) which reabsorb the filtrated urinary glucose and to prevent glucose excretion [32]. SGLT2 inhibitors (SGLT2i) are a class of medications which specifically inhibit these active carriers and thereby induce urinary glucose excretion leading to lower levels of glycemia in patients with diabetes [5]. This potent oral glucose lowering

[2] SHC-transforming
[3] adapter protein with a PH and SH2 domain
[4] insulin receptor substrate type 1
[5] extracellular signal-regulated kinase 1/2
[6] protein kinase C
[7] Ribosomal protein S6 kinase beta-1
[8] serine/threonine-protein kinase 2
[9] mammalian target of rapamycin
[10] Rho-associated protein kinase 1
[11] AMP-activated protein kinase
[12] Glycogen synthase kinase 3
[13] phosphoinositide 3-kinase
[14] Phosphatidylinositol 4,5-bisphosphate
[15] Phosphatidylinositol 3,4,5-trisphosphate

agents work entirely independent of insulin and are dependent on blood glucose levels so that they have a lower risk of hypoglycemia [33].

Since the discovery of phlorizin as the first SGLT2i in the early of 1980s, several forms of these agents have introduced which all reduce the blood glucose below the threshold of tubular transporter for glucose reabsorption [9, 33-35]. However, in addition to its anti-hyperglycemic effects through glycosuria, they may provide other beneficial effects such as suppression of gluconeogenesis, improving insulin sensitivity in peripheral tissues, increasing glucagon response and stimulation of insulin secretion from the beta cells of pancreatic islets [8, 36-38]. Canagliflozin, dapagliflozin, and empagliflozin are the known forms of this class of antidiabetic drugs [39]. The side effects of SGLT2i include dehydration, dizziness, hypotension and genito-urinary infections [39].

GLT2i and iinsulin sensitivity

Emerging evidence indicates that SGLT2 inhibition can interfere with various steps of IST and modulate insulin sensitivity in peripheral tissues [7, 8, 36]. In the following sections, we present the main involved molecular mechanisms (Table 1).

1. **Inhibition of glucose toxicity**

SGLT2i promotes excretion of glucose through urine, thereby lowering blood glucose and glucotoxicity [9]. Chronic hyperglycemia results in glucotoxicity as seen in the diabetic milieu [40-42]. This toxic milieu negatively affects beta-cell function and is closely related to insulin resistance [43]. On the other hand, strong evidence suggests that SGLT2i-induced glycosuria potentially improves glucotoxicity [44].

Mervoric et al. in 2014 has shown that SGLT2 inhibition by dapagliflozin reduces glucotoxicity and thereby improves glycemic control in the diabetic milieu [7]. Two weeks of dapagliflozin therapy in men with diabetes improved glucotoxicity by lowering the fasting blood glucose and increasing tissue sensitivity to insulin [7]. These anti-glucotoxicity effects of SGLT2i are reversible after treatment cessation [7]. List and coworkers in 2011 provided further evidence suggesting that SGLT2i therapy induces insulin sensitivity, beta-cell activity and morphology preservation via ameliorating the glucotoxicity [43]. Kern and colleagues emphasized this finding in 2016, where they found that SGLT2i therapy in diabetic mice improved insulin signaling, leading to better glucose homeostasis [8]. These reports suggest that attenuation of toxic effects of glucose by SGLT2i is one of the main molecular pathways in which they can improve insulin sensitivity [7, 8, 43].

2. Caloric disposition and weight loss

Caloric disposition is an established effect of SGLT2i therapy since they promote urinary glucose excretion [45]. Caloric disposition can translate to weight loss as well as a reduction in the circulating lipids [45-47]. It has been demonstrated that SGLT2i therapy can reduce body weight in patients with diabetes [48]. On the other hand, it has been well confirmed that lower mass of adipose tissue and lower levels of circulating lipids are correlated to an increase in insulin sensitivity [49]. The role of inflammatory responses in insulin resistance is completely verified and patients with diabetes have relatively higher levels of circulatory inflammatory mediators [50, 51]. The central part of these inflammatory responses is mediated by adipocytes and their related active mediators, known as adipokines [50]. These active biologic adipocyte-derived cytokines have prominent roles in insulin resistance [52]. There are strong animal and human evidence indicating that high-fat diet and obesity are one of the main underlying causes

of insulin resistance and the onset of DM [53, 54] which SGLT2i therapy can potentially reverse [55]. For example, Shamansurova et al. in 2016, on a diabetic mouse, demonstrated that depletion of adipose tissue improved insulin sensitivity by improving locomotor activity and basal metabolism. Furthermore, increasing adiponectin which is another form of adipocytokines, is associated with insulin sensitivity [56]. Since lesser adipocytes can potentially improve insulin sensitivity, weight loss is strongly recommended in patients with T2DM [56].

Xu et al. in 2018 demonstrated that "visceral fat lowering" effect of SGLT2i can markedly improve the insulin sensitivity by ameliorating the lipotoxicity [57]. They reported that SGLT2i therapy in patients with diabetes could attenuate insulin resistance by lowering the rate of adipocyte-induced inflammatory responses by regulating energy homeostasis and a reduction in lipotoxicity-induced insulin resistance [57]. Empagliflozin (SGLT2i) increases insulin sensitivity by ameliorating inflammatory processes and visceral fat lowering via an M2 macrophage polarization-dependent mechanism in white adipose tissue (WAT) of diabetic mice. Also, it promoted browning adipose tissue led to increased energy expenditure and thermogenesis[58]. Xu and colleagues provided evidence for the effects of SGLT2i on WAT browning and fat utilization induced by activating M2 macrophage. Thus, they concluded that empagliflozin has beneficial effects on obesity-related inflammation and insulin resistance. [57]. In a clinical trial with 38 diabetic patients, SGLT2i therapy by canagliflozin significantly improved insulin resistance by a reduction in body weight as well as lowering visceral and ectopic fatty tissues in Japanese patients with T2DM [59]. Furthermore, Singh and coworkers in 2019 provided the same evidence about Indian patients with T2DM suggesting that visceral fat lowering effect of SGLT2i therapy potentially improves insulin sensitivity [60]. Okamoto et al. in 2016 found that dapagliflozin improved adipocyte function which increased adiponectin in obese patients with T2DM [61]. Matsumura and coworkers in 2017 also reported

that canagliflozin increased adiponectin-induced glucose homeostasis in patients with T2DM [62]. Tobita et al in 2017 demonstrated that canagliflozin therapy in patients with T2DM improved glucose metabolism by reducing visceral fat resulting in metabolic improvement [63]. Garvey et al. in 2018 provided more evidence implying that canagliflozin improved glucose homeostasis by improving lipid metabolism and leptin level in patients with diabetes [64]. This evidence strongly suggests that SGLT2i therapy can increase insulin sensitivity and improve glucose homeostasis at least partly via lowering adipocytes number, fatty tissues and plasma lipids [57, 59, 60].

3. Attenuating the inflammatory responses

Inflammatory responses are potent upstream events in the pathophysiology of DM, contributing to insulin resistance and lowering insulin sensitivity in the peripheral tissues [52]. Patients with diabetes have higher levels of inflammatory mediators, including active cytokines in the blood, which potentially impair IST, thereby reducing insulin sensitivity [52]. Inflammation hypothesis states that inflammation is strongly involved in the pathophysiology of DM [65, 66]. There is growing evidence that SGLT2i can inhibit or decline inflammatory processes and prevent the expression of inflammatory mediators [67, 68]. It has been suggested that SGLT2 inhibitors can suppress inflammatory responses via both direct (impact on the immune system) and indirect molecular pathways via at least 5 different molecular mechanisms such as modulation of the renin-angiotensin system (RAS), altering hemodynamic changes, reduction in obesity-induced inflammation, modulation of immune system function and readjustment of the redox state in tissue leading to a lower rate of inflammatory responses [57, 69-74]. Since lower inflammatory response can be translated to a reduced impairment in IST and lower risk for insulin resistance, we concluded that anti-inflammatory properties of SGLT2i could be exploited to design novel therapeutic approaches for patients with diabetes.

4. Improvement in beta-cell function

Majority of patients with T2DM have some impairment in pancreatic beta-cell function [75, 76]. They have dysfunctional islets with suboptimal response to stimuli and thereby, inadequate insulin release [76]. The number of functional beta cells is also reduced due to pathophysiological mechanisms such as oxidative damage, fibrosis and inflammation leading to higher rates of apoptotic processes [76, 77]. Improving beta cells function is one of the main targets in patients with diabetes to normalize hyperglycemia [78, 79]. Although one may conclude that SGLT-2i can only increase insulin action, there direct evidences suggesting SGLT2 inhibition increases insulin sensitivity by improving islet cell function in diabetic milieu [55, 80-82]. This action could be possibly through improvement in beta cell-mediated insulin dependent glucose homeostasis.

Jurczak et al. in 2013 demonstrated that SGLT2 deletion in animals with diabetes improves glucose homeostasis and enhances insulin sensitivity by preserving the beta cells function in a diabetic milieu [55]. They have observed a 60% increase in beta cell mass and lower levels of apoptotic death leading to more functional pancreatic islets in SGLT2 knockout diabetic mice [55]. Kaneto and colleagues in 2017 observed that SGLT2i therapy protects beta cells against glucose toxicity and β-cell lipotoxicity as well as improves islet cell function leading to increased insulin sensitivity [80]. SGLT2 inhibitory agents can preserve islet function by attenuating the pathophysiologic pathways and deleterious molecular mechanisms [80]. They can suppress glucotoxicity, lipotoxicity, inflammatory processes, fibrotic processes and oxidative damages leading to lower rates of beta cell death and more functional mass of islets thereby improving insulin sensitivity and glucose homeostasis [79-81, 83]. Cheng et al. in 2016 surveyed the potential beneficial effects of empagliflozin and found that it improved glucose homeostasis by preserving the beta-cell function probably via suppressing the glucotoxicity-

induced oxidative stress-dependent beta-cell death and by increasing regeneration of pancreatic islet cells in diabetic mice [83]. Also, Asahara et al. in 2019 suggested that SGLT2i re-adjust glucose homeostasis by preserving beta cells function [81].

Merovci et al. in 2015 in a human clinical trial demonstrated that SGLT2i with dapagliflozin increased insulin sensitivity by an improvement of beta cells function in T2DM individuals [84]. Al Jobori et al. in 2018 reported that empagliflozin therapy for two weeks is associated with better beta-cell function in patients with diabetes [82]. They showed that empagliflozin therapy increased islets' glucose sensitivity during periods of hyperglycemic clamps which is a method for assessing the function of beta cells [82]. Moreover, Takahara and coworkers in 2015 evaluated the islets function using disposition index and found that four weeks of treatment with ipragliflozin improved beta-cell function in patients with T2DM [85]. These strong experimental and human evidence firmly suggest that SGLT2i therapy improves beta cell function in a diabetic milieu [81-83, 85]. They demonstrated that SGLT2i therapy in diabetic milieu is a potential way for improvement of beta-cell function via several mechanisms leading to an increased number of functional beta-cells and thereby more ability to secrete insulin in response to circulating glucose [81-83, 85].

5. Reduction in oxidative stress

Free radical generation is a common phenomenon in the biologic milieu which has important biologic functions such as cellular signaling in the physiologic range [86]. In high concentrations, free radical species produces oxidative stress which is one of the main and potent upstream events in various pathophysiologic states including DM [87]. Oxidative stress impairs IST significantly and reduces insulin sensitivity, thereby increasing insulin resistance leading to DM [88, 89]. It negatively affects peripheral insulin sensitivity and down-regulates

glucose transport system in insulin-dependent cells [88]. Alleviating the free radical production and normalizing its concentration near to the physiologic ranges is one of the main therapeutic targets in DM and preventing its complications [90, 91].

There is growing evidence that SGLT2i improve redox state in the diabetic milieu [92]. They can exert potent antioxidative properties by several molecular mechanisms including improvement in mitochondrial dysfunction [92, 93], regulating the RAS (renin-angiotensin system) activity [70, 94], attenuating inflammation-induced free radical generation [95, 96], lowering the AGEs (advanced glycation end products) and AGE/RAGE (receptors for advanced glycation end products) crosstalk [97, 98], down-regulating the pro-oxidant enzymes expression/activity as NADPH (Nicotinamide adenine dinucleotide phosphate) oxidase, eNOS (endothelial nitric oxide synthase) and xanthine oxidase [99, 100]. Based on this evidence, SGLT2 inhibition ameliorates oxidative stress and could improve insulin sensitivity by attenuating oxidative damages in the diabetic milieu [57, 101].

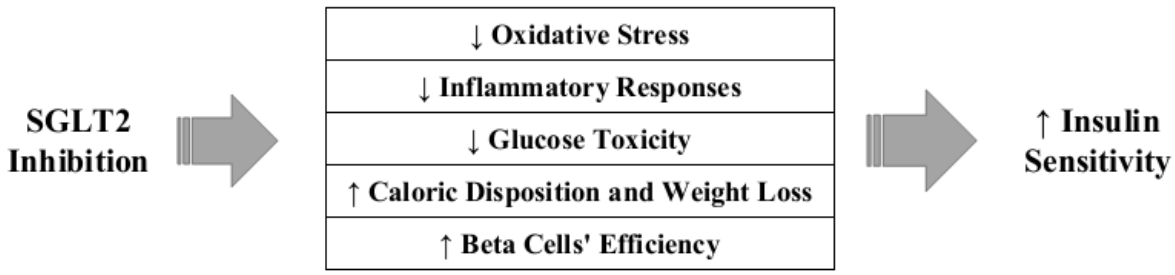

Figure 1: possible pathways by which SGLT2i induce insulin sensitivity

Conclusion

Sodium-glucose co-transporter inhibitors are a relatively new class of medications used in the management of diabetes for lowering blood glucose as well as reducing microvascular and macrovascular complications associated with it. Beyond the glycosuric effects, these agents have pleiotropic effects on glucose homeostasis (Table 1). While they are considered as potent glucose-lowering drugs, they can also improve insulin sensitivity and glucose homeostasis via several molecular pathways including reduction of glucotoxicity and lipotoxicity, improvement in beta cell function, reduction of oxidative damages and inflammatory processes as well as induction of caloric disposition and weight loss (Fig 1).

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Conflict of Interests

All authors declare that they do not have any conflict of interest in this study.

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None.

Table 1. Main molecular mechanisms by which SGLT2i therapy increase insulin sensitivity (IST=insulin signal transduction)

Molecular Mechanisms	Detailed Effects of SGLT2i	Ref.
Inhibition of Glucose Toxicity	Normalize hyperglycemia near to physiologic ranges leading to lowering rates of glucotoxicity and thereby increasing peripheral insulin sensitivity	[7, 8, 43]
Caloric Disposition and Weight Loss	Induce caloric deposition by glycosuria leading to lesser adipocytes number as well as lesser adipocytokines and lipotoxicity thereby improving peripheral insulin sensitivity	[57, 59, 60]
Attenuating the Inflammatory Responses	Induce peripheral insulin sensitivity by reduction of various inflammatory processes	[58, 68, 70, 102-106]
Improvement in Beta Cell Function	Improve beta cell mass/function by ameliorating the pathophysiological pathways involved in the islets cells' death	[55, 79-85]
Reducing the Oxidative Stress	Reduce insulin resistance by attenuating the oxidative stress-dependent IST impairment	[57, 70, 89, 94, 97, 98, 101]

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