Chapter

SGLT2 Inhibitors Therapy in Type 2 Diabetes Mellitus

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Abstract

Type 2 diabetes mellitus (T2DM) is a chronic progressive disease characterized by chronic hyperglycemia and increased risk of cardiovascular disease (CVD). It results from multiple defects that lead to defective regulation of the blood glucose and requires continuous medical care with multifactorial risk-reduction strategies beyond glycemic control. Multiple groups of drugs have been approved in the past decades that work through different mechanisms. Apart from their limited efficacy in reducing cardiovascular outcome, most of them are neutral, and some may even increase mortality from CVDs such as rosiglitazone. The kidney has an important role in glucose regulation that was only recently targeted for drug development. Sodium-glucose cotransporter 2 inhibitors (SGLT2-I) are a new class of oral anti-hyperglycemic (OAH) agents that mainly act by preventing the reabsorption of filtered glucose by renal convoluted tubules. By their insulin-independent unique mechanism of action, SGLT2-I result in treating hyperglycemia while avoiding hypoglycemia, promote weight loss, reduce blood pressure, and, more importantly, decrease the risk of major adverse cardiovascular events (MACE). Therefore, SGLT2-I address fundamental aspects of the unmet needs of T2DM management that most of the other OAH failed to resolve. The main side effects of SGLT2-I are slight increase in the incidence of genital mycotic infections (GMI) and euglycemic ketoacidosis (EKA) along with increased risk of lower limb amputations, which has been reported with some but not all agents of this class.

Keywords: type 2 diabetes mellitus, SGLT2 inhibitors, hyperglycemia, cardiovascular disease, mycotic infections, euglycemic ketoacidosis

1. Introduction

T2DM is a chronic progressive metabolic disease characterized by chronic hyperglycemia and increased risk of CVDs that result from defective regulation of the blood glucose and requires continuous medical care with multifactorial risk-reduction strategies beyond glycemic control [1].

The magnitude of the problem can be assessed from the report of International Diabetes Federation (IDF) Atlas, where it was estimated that in 2017 there were 451 million people (age 18–99 years) with diabetes mellitus (DM) worldwide and that almost half of all people (49.7%) living with DM are undiagnosed [2]. More alarmingly, the projected figures for the prevalence of DM according to the same IDF report are expected to increase to 693 million by year 2045. In addition, there

was an estimated 374 million people with impaired glucose tolerance (IGT), and in 2017 sadly, approximately 5 million deaths worldwide were attributable to DM. The global healthcare expenditure on people with DM was estimated to be USD 850 billion in 2017 [2]. Therefore, it is obvious that T2DM comes with a huge burden of morbidity and mortality and this is mainly due to the development of diabetes-specific microvascular complications and accelerated atherosclerotic macrovascular disease [3, 4].

T2DM is the seventh leading cause of death in the United States, and the estimate of the World Health Organization that T2DM-related mortality is expected to double in number by year 2030 if not treated properly further raises the alarm [5].

Improving glycemic control in people with DM not only substantially reduces their risk of microvascular complications and CVDs but also ameliorates the metabolic dysfunctions that contribute to the progressive nature and course of the disease. Evidence from United Kingdom Prospective Diabetes Study (UKPDS) showed that 1% reduction in glycosylated hemoglobin (HbA1C) was associated with relative risk reduction of 14% in fatal and nonfatal myocardial infarctions, 12% in fatal and nonfatal stroke, and 16% in heart failure [6].

While intensive glycemic control has been shown to substantially reduce the risk of microvascular complications, its value in reducing macrovascular complications that was previously reported 20 years after the end of UKPDS has recently been put in doubt after the ACCORD and ADVANCE Trials, which either showed increased risk of death or no benefit [7–9].

The UKPDS was the first study to show unequivocally that in patients with newly diagnosed T2DM, lowering blood glucose with intensive therapy to a median HbA1C of 7.0% was associated with 25% reduction in the rate of microvascular complications [7].

Moreover, after 10 years of follow-up post UKPDS, the benefits continued with regard to reduction in microvascular complication, and the reduction in macrovascular events was clearer [10].

Notable are the results of ACCORD, ADVANCE, and the Veterans Affairs Diabetes Trial (VADT) studies in patients with advanced T2DM, and either known CVD or multiple CVD risk factors showed that lowering blood glucose (HbA1C levels 6.4–6.9%) delayed the onset or slowed the progression of microvascular complications, but there was no significant reduction in CVD outcomes [8, 9, 11]. On the other hand, the ACCORD study suggests that less intensive therapy may be more appropriate in patients with T2DM and high risk of CVDs because intensive therapy to target HbA1C levels (6.4–7.5) was associated with a 22% increased risk of all-cause mortality [9].

Based on what we have learned from these studies, the American Diabetes Association and the European Association for the Study of Diabetes guideline suggest reducing HbA1C levels to around 7%, but in younger patients with short duration of diabetes and no significant heart diseases, HbA1C levels can be reduced to less than 6.5%. In older patients and those with advanced CVD and limited life expectancy, less stringent HbA1C levels around 8% may be appropriate [3].

Hypoglycemia, weight gain, and progressive beta-cell failure are the major limiting factors for intensive glycemic control approach and in achieving the proposed HbA1C goals [12].

The efficacy of the available OAHs and their effectiveness in the management of T2DM were reported in 2013 according to which and despite availability of several therapeutic options, 33–49% of patients fail to meet the targets for control of glycemia, blood pressure, or cholesterol and only a minority, around 14%, were able to meet targets for all three measures [13].

Apart from the limited efficacy of some OAHs in reducing CV risk, most of these agents are neutral when it comes to CVD risk reduction and some may even increase mortality from CVDs.

Therefore, for some time, T2DM unmet needs remained unresolved, and the need for innovation continued. For some experts in the field, it was suggested that newer OAHs should be so unique in their properties, namely, addressing the unmet needs and filling the gaps of the available OAHs such as weight gain, hypoglycemia, and CV safety to pass the test of FDA approval after the rosiglitazone story which was withdrawn from the market in 2008 because of its association with increased risk of CVDs [14]. Following rosiglitazone incident, the FDA mandated cardiovascular outcome trials on all newer OAH agents [15].

T2DM treatment requires individualized management with consideration of a number of patient factors. These include the degree of HbA1c reduction needed, risk of hypoglycemia, the side effect profile of medications, comorbid medical conditions, and the ability of patients to adhere to the medication regimen along with their preferences. Development of novel drugs with newer and complimentary mechanisms of action is needed to address the unwanted side effects and limitations of most of the old OAH agents, namely, the risk of hypoglycemia, weight gain, durability, and CV safety profile. Availability of newer medications with such profiles will simplify therapy and enhance patient adherence, especially in this era of increasing obesity.

Among newer classes of drugs, SGLT2-I hold great promise, and several agents from this group have already been approved by the US FDA and elsewhere for treatment of T2DM. They have a novel therapeutic mechanism of action when compared with other drugs available for T2DM management. The main site of action of SGLT2 inhibitors is in kidneys—a site which plays a major role in glucose homeostasis and has never been explored before.

2. Glucose homeostasis

Glucose is an essential and principal fuel source for cellular metabolism in the human body and is the main energy resource for the central nervous system, muscles, and fat—and insulin plays a key role in its effective utilization. The glucose homeostasis is rapidly adjusted in response to physiological changes such as food intake, exercise, and acute stress, and its blood level is adjusted by control of absorption of glucose, its metabolism in the liver, its excretion by kidneys, and uptake into muscles and adipose tissue. Functions of various proteins associated with regulation of glucose metabolism and homoeostasis get affected by diseases such as DM and hepatic disorders [16].

2.1 Handling of glucose in the intestine

After ingestion of carbohydrate-rich diet, blood glucose is regulated in response to the increase in glucose concentration in the intestinal lumen and in response to the increase in blood glucose level. The increase in glucose levels in the lumen of the small intestine provides a signal for the upregulation of intestinal glucose absorption [17] and leads to secretion of gut hormones such as glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), which increase the glucose-dependent stimulation of insulin secretion from the pancreatic β-cells and also influence appetite [18]. Insulin reduces glucagon secretion by acting on pancreatic alpha cells and also reduces blood glucose level by increasing glucose uptake in fat and muscle cells and changing glucose metabolism in the liver [1].

2.2 Glucose transport across plasma membrane

Being a highly polar molecule, glucose is unable to cross the lipid bilayer of the plasma membrane of all living cells; therefore, transport proteins within the cell membrane are required to facilitate glucose transport from the extracellular to the intracellular space.

Two distinct groups of glucose transporters belonging to solute carrier gene series (SLC) containing more than 50 transporter families have been described [19]. The first one is facilitated glucose transporters (GLUT) which is encoded by the SLC2 family of transporters GLUT1–4 and GLUT6–12, which help in passive transportation of glucose from the extracellular to the intracellular space along its chemical gradient without consuming any energy and equilibrate glucose concentration on both sides of membrane. The other one is sodium-glucose cotransporters (SGLTs) which are encoded by SLC5 family of transporters SGLT1–6 which actively transport glucose across plasma membranes against its concentration gradient. This process requires energy which is provided by simultaneous coupled transportation of sodium along its concentration gradient [19]. In this way, SGLTs help in concentrating glucose inside the cells.

The two principal sites of action of SGLTs are the intestine and kidney where they mediate glucose transportation across the intestinal lumen and the epithelial cells in the proximal renal tubules, respectively [20].

2.3 Handling of glucose by the kidney

The kidney plays an important role in glucose homeostasis by the process of filtration and reabsorption. Renal glomeruli filter approximately 180 liters of plasma daily which translates into filtration of approximately 180 g of glucose. In normal healthy subjects, all of this glucose is reabsorbed completely so that virtually no glucose is excreted in urine. Around 90% of the filtered renal glucose is reabsorbed in early part (S1) of proximal convoluted tubules by low-affinity high-capacity SGLT2. High-affinity low-capacity SGLT1 in distal straight segment (S3) of the proximal tubules reabsorbs the remaining 10% of the filtered renal glucose [21].

This process of glucose reabsorption is achieved by active Na^+ removal at basolateral surface by the Na^+/K^+-ATPase which generates the electrochemical driving force for apical glucose entry via Na^+-driven SGLTs. Reabsorbed glucose exits from the basolateral surface of the cells along its concentration gradient primarily via GLUT2 and reenters the bloodstream [21].

When blood glucose level exceeds 200 mg/dL, the excess glucose starts appearing in urine as renal transport maximum (T_m) of glucose is reached. The blood glucose level at which T_m is reached is called threshold and is around 300 mg/dL in healthy nondiabetic individuals, but glucose starts appearing in urine at around 200 mg/dL due to heterogeneity of individual nephrons in their T_m property and mismatch between glomerular filtration and tubular reabsorption of glucose. This safety valve-like action prevents extreme hyperglycemia [22].

The T_m of the proximal tubules on average is around 375 mg/minute although it shows inter-individual variations. The filtered glucose load is directly proportional to blood glucose concentration. In normal nondiabetic individuals, the filtered glucose load is less than 375 mg/minute; therefore, the entire amount is reabsorbed, and their urine is free of any glucose. This process has survival benefits as it allows the kidneys to conserve glucose and can be viewed as an adaptive mechanism. In patients with T2DM, the filtered load may exceed 375 mg/minute; therefore, the T_m is exceeded, and all glucose in excess of the T_m spills over in urine [21]. In T2DM subjects, this adaptive mechanism becomes maladaptive. The increased expression of SGLT1/SGLT2 occurring in DM subjects results in increased renal

glucose reabsorption which ultimately leads to maintenance of a state of persistent hyperglycemia [23, 24]. The tubular growth leads to increased T_m for glucose which further exacerbates hyperglycemia [22].

Based upon these facts, the contribution of the kidney in development and maintenance of state of hyperglycemia in T2DM is quite evident. Therefore, SGLT2-I provide a pathophysiologically rational and novel approach to its treatment.

3. Clinical effects of mutations in SGLT1 and SGLT2

Autosomal recessive mutations in SGLT1 lead to a disorder called glucose galactose malabsorption (GGM). SGLT1 becomes nonfunctional, and infants with GGM develop severe watery diarrhea, dehydration, and metabolic acidosis that cease on diet free of glucose, galactose, and lactose [25].

In 1927, the first case of familial renal glycosuria was reported, in which a mutation in gene for SGLT2 resulted in loss of glucose in urine ranging from 1 to 150 g per 1.73 m^2 body surface areas per day. Almost 50 mutations have been identified so far leading to this condition which is characterized by urinary glucose excretion in the presence of normal plasma glucose levels and an absence of signs of general renal tubular dysfunction. Patients have normal oral glucose tolerance test and usually present with osmotic symptoms without any serious complications [26, 27]. The condition is not associated with any change in intravascular volume, serum glucose levels, or renal or bladder dysfunctions. These patients do not show higher incidence of kidney disease, DM, or urinary tract infections although those affected with severe forms of the disease may demonstrate activation of renin-angiotensin-aldosterone axis as indirect evidence of volume contraction [20, 26, 27].

These observations further strengthen the belief that SGLT2-I could potentially be developed as safe OAH.

4. Sodium-glucose cotransporters

So far, six different SGLTs have been described; however, apart from SGLT1 and SGLT2 which are well characterized, little is known about the function and clinical significance of the others [20]. Both SGLT1 and SGLT2 are large-membrane proteins consisting of 670 amino acids, and each has 14 transmembrane helical domains. The homology between SGLT1 and SGLT2 is around 58% (**Table 1**) [20].

Transporter	Gene	Substrate	Distribution and localization
SGLT1 (SLC5A1)	22q12.3	Glucose, galactose	Intestine, trachea, kidney, heart, brain, testis, prostate
SGLT2 (SLC5A2)	16p12.p11	Glucose	Kidney, brain, liver, thyroid, heart, muscle
SGLT3 (SLC5A4)	21q22.12	Glucose	Intestine, testis, uterus, lung, brain, thyroid
SGLT4 (SLC5A9)	1p32	Glucose, mannose	Intestine, kidney, liver, brain, lung, trachea, uterus, pancreas
SGLT5 (SLC5A10)	17p11.2	Glucose, galactose	Renal cortex
SGLT6 (SLC5A11)	16p12.1	D-chiro-inositol	Spinal cord, kidney, brain

Table 1.
Genes, substrates, and distributions of SGLT [28].

4.1 Sodium-glucose cotransporter 1 (SGLT1)

4.1.1 Locations of SGLT1

Studies have classically localized SGLT1 to the small intestine and the kidney where their pathophysiological roles are known in details [28].

Various other body organs such as the heart, lung, trachea, liver, skeletal muscle, gall bladder, rectum, colon, brain, blood vessels, breast, uterus, testis, and pancreatic alpha cells have shown mRNA level expression of SGLT1 [28–33].

4.1.2 Functional properties of SGLT1

SGLT1 transports one molecule of glucose or galactose together with two sodium ions. This stoichiometric ratio of 1:2 enables to raise the intracellular glucose level orders of magnitude above the corresponding extracellular concentration. The apparent Michaelis-Menten Km values for glucose and galactose at physiological extracellular sodium concentration and membrane potential are 0.5 and 1.0 mM, respectively. It has high affinity for both glucose and galactose but has a lower transport capacity (T_{max} = 2 nmol/mg protein per minute) [28, 34].

4.1.3 Physiological functions of SGLT1

SGLT1 is highly expressed in the small intestine and is located in the brush border membrane (BBM) of the enterocytes and in endocrine cells of gut and the K- and L-cells which secrete GIP and GLP-1/GLP-2, respectively. SGLT1-mediated translocation of glucose is the rate-limiting step for small intestinal glucose absorption [17, 28, 29]. Absorbed glucose in the enterocytes is released across the basolateral membrane and enters blood circulation via GLUT2. During bacterial infection, SGLT1 protects the small intestine from lipopolysaccharide-induced inflammation because of high luminal glucose concentrations [35].

In the kidney, SGLT1 is located at BBM of S3 segment of renal tubules and is responsible for the first and rate-limiting step in reabsorption of glucose which escaped SGLT2-mediated reabsorption in S1 and S2 segments. In normal healthy adults, it only absorbs around 10% of the filtered glucose load, but in DM patients with uncontrolled hyperglycemia, the fraction of SGLT1-mediated renal glucose absorption increases significantly. Similarly, in patients on SGLT2-I therapy, the fraction increases to around 50–70% [17, 28, 29]. SGLT1 may play a protective role during treatment with nephrotoxic drugs such as cisplatin [36].

SGLT1 mRNA has been detected in the frontal cortex, hypothalamus, and Purkinje cells of cerebellum and hippocampus in brains of human, rabbit, and rat [33, 37, 38]. It is mainly localized in the luminal membrane of the endothelial cells, and its location and functional activity suggests a pivotal role in securing energy supply to neurons during conditions of increased energy and glucose demand such as hypoxia and/or hypoglycemia. SGLT1-mediated neuronal glucose uptake is involved in glucose-induced neurotoxicity during ischemic stroke [39].

SGLT1 is located at the myocyte sarcolemma and in small blood vessels of the heart [29, 32]. SGLT1-mediated glucose uptake is of clinical significance as it leads to ATP generation by glycolysis during myocardial ischemia and/or hypoglycemia [40]. At the same time, it may increase toxic effects that are mediated by generation of reactive oxygen species (ROS) during hyperglycemia [41].

SGLTI1 mRNA has been detected in the lung, trachea, and bronchi, and its protein has been localized to alveolar type 2 cells and to the luminal membrane of bronchiolar Clara cells by immunohistochemistry [30, 31]. SGLT1-mediated

glucose absorption contributes to fluid absorption and may provide energy for surfactant production in alveolar type 2 cells as well as for mucin and surfactants in Clara cells [42].

Similarly, the human gall bladder and liver have shown the presence of SGLT1 mRNA [29, 30]. Its expression at mRNA and protein level has been demonstrated in human pancreatic alpha cells; however, little is known about its functional role [30, 31]. SGLT1 has also been expressed in activated T-lymphocytes of mice where it may have a possible role in immune reactions [43].

4.1.4 Regulation of SGLT1 expression

The complex process of regulation of activity and expression of SGLT1 occurs in a tissue-specific manner. In the small intestine, upregulation of SGLT1 expression occurs in response to high-salt and/or high-glucose diet through transcriptional regulation which is also responsible for the circadian periodicity of SGLT1 expression [44, 45]. Its expression gets upregulated in the small intestine in diabetics [46] and in response to bacterial infections [35], while downregulation of SGLT1 expression occurs during chronic intestinal inflammation [47].

4.2 Sodium-glucose cotransporter 2 (SGLT2)

4.2.1 Locations of SGLT2

In humans, SGLT2 is strongly expressed in the kidney where it has been localized to the brush border membrane of the S1 and S2 segment of the proximal tubules. On the other hand, SGLT1 has been localized to the brush border membrane of the S3 segment of proximal convoluted tubules of the kidney [28–30, 48].

Proteins and mRNA of SGLT2 have also been found in alpha cells of the pancreas [31]. In addition to the kidney and pancreas, small amount of SGLT2 mRNA have been identified in the testis, liver, lung, and cerebellum [21, 28–30, 48].

4.2.2 Functional properties

SGLT2 is highly selective for glucose over galactose. It has low affinity for glucose with Km = 2 mM but with high transport capacity with T_{max} = 10 nmol/mg protein per minute and operates with a 1:1 stoichiometry of sodium and glucose. The apparent Michaelis-Menten Km values for glucose and sodium in human SGLT2 are 5 and 25 mM, respectively [28].

4.2.3 Physiological functions of SGLT2

4.2.3.1 Functions of SGLT2 in the kidney

Details of the physiological functions of SGLT2 in the kidney have already been mentioned earlier in Section 2.3.

In T2DM, SGLT2-mediated reabsorption of glucose and sodium is increased and can be considered physiologically maladaptive as it prevents an increase in urinary glucose excretion at high blood glucose levels. The increase in proximal tubular sodium reabsorption leads to fall in the distal tubular sodium and chloride concentrations which result in glomerular hyperfiltration [49] and plays a central role in the development of diabetic nephropathy [50].

The triad of hyperglycemia, elevated GFR, and the increased proximal tubular glucose reabsorption altogether leads to increase in kidney size and volume which is

combined with glomerular hypertrophy, enlarged proximal tubules, inflammation, and interstitial fibrosis. These hyperglycemia-induced alterations lead to micro- and macroalbuminuria which culminate into renal failure [49].

4.2.3.2 Functions of SGLT2 in pancreatic alpha cells

During fasting when blood glucose level is low, several counter-regulatory responses are generated to increase and maintain blood glucose within normal range. Pancreatic alpha cells secrete glucagon which stimulates glycogenolysis and gluconeogenesis in the liver. Conversely, glucagon secretion is inhibited when blood glucose level increases after taking food [51]. Inhibition of glucagon secretion is mediated by paracrine effect of insulin and direct glucose-mediated regulation of glucagon secretion. In alpha cells, SGLT2-mediated glucose uptake is a critical step involved in direct regulation of glucagon secretion [31].

The expression of SGLT2 at mRNA level increases in alpha cells in obesity and prediabetes. Once T2DM develops, the glucotoxicity leads to decrease in its expression. The glucagon secretion blockage which normally occurs at high plasma glucose levels gets blunted due to downregulation of SGLT2 causing enhanced endogenous glucose production in the liver which further aggravates hyperglycemia [31].

4.2.4 Regulation of SGLT2

SGLT2 gene is located at chromosome 16 p11.2 and is expressed primarily in renal cortex. Various transcription factors are involved in regulation of SGLT2 such as SP-1, HNF1-alpha, and HNF4A, and their binding sites have been identified on SGLT2 promoter region [28].

High-sodium intake promotes urinary sodium and glucose excretion by increasing plasma adiponectin level through stimulation of peroxisome proliferator-activated receptor delta in adipose tissue. The enhanced adiponectin downregulates SGLT2 leading to reduced reabsorption of sodium and glucose. Due to hyperglycemia, this mechanism gets dampened in DM. Binding of SP-1 and HNF1-alpha at the promoter site is involved in this regulation [52], while HNF4A participates in glucose-dependent regulation of SGLT2 in alpha cells of the pancreas [31].

Activation of transcription factor NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) downregulates transcription of SGLT2 in the presence of hyperglycemia due to increase in ROS [53]. Sympathetic innervation has been found to be involved in transcriptional upregulation of SGLT2 in the kidney [54].

Posttranscriptional regulation of SGLT2 is yet to be understood well. Recently, it was found that the 17 kDa protein membrane-associated protein 17 (MAP17) upregulates functional activity of SGLT2 in the plasma membrane [55].

5. Development of SGLT inhibitors

Given the findings discussed in the above sections and considering the physiological functions of SGLT1 and SGLT2, it was an obvious idea to use SGLT1 and SGLT2 inhibitors as OAHs. Targeting hyperglycemia by inhibiting intestinal and renal glucose reabsorption appeared to be a novel therapeutic strategy.

Phlorizin was discovered around 150 years ago, which is a chemical found in the root bark, leaves, shoots, and fruit of the apple tree, and soon thereafter it was found to increase renal glucose excretion in healthy human beings. Phlorizin is a naturally occurring competitive nonselective inhibitor of SGLT1 and SGLT2.

In 1987, it was reported that subcutaneous phlorizin administration normalized plasma glucose profiles in insulin-resistant diabetic rats along with improving insulin sensitivity [56].

However, due to poor water solubility and poor oral bioavailability as it is metabolized to phloretin by glucosidase in gut and unselective SGLT1 and SGLT2 inhibition, phlorizin was not an ideal therapeutic agent. It has low selectivity for SGLT2 compared to SGLT1.

T-1095 was the next agent developed but did not continue into clinical development as it was again nonselective in nature and had safety concerns [57].

By modification in basic structure of phlorizin, other SGLT2-I were developed, including AVE-2268, remogliflozin, sergliflozin, and WAY-123783.

All of them have the glucoside moiety linked to a distal phenolic ring via an O-linkage. Due to susceptibility of O-linkage to degradation by β-glucosidases which reduced their utility, development of more metabolically stable C-linkage SGLT2 inhibitors was prompted with focus on increasing selectivity for SGLT2 versus SGLT1. This led to discovery of dapagliflozin, canagliflozin, empagliflozin, ipragliflozin, BI 44847, and LX 4211 [58, 59].

6. Clinical effects of SGLT2 inhibitors in diabetes mellitus

Currently, there are seven SGLT2 inhibitors approved for clinical use. They are given orally and absorbed by the intestine. Due to higher selectivity for SGLT2 versus SGLT1, inhibition of intestinal SGLT1 can be avoided, though it is still possible at high oral doses. At pharmacological doses, their serum levels achieved are too low to inhibit SGLT1 in other organs (**Table 2**).

Clinical effects observed for different SGLT2-I will be described and discussed together for the sake of clarity.

6.1 Effects on diabetes and metabolism

In T2DM, upregulation of SGLT2 expression increases its T_m by around 20%. SGLT2-I is filtered in glomeruli and inhibits glucose reabsorption in S1 segment of proximal tubule leading to a reduction of 30–50% in T_m of SGLT2 [67].

In the presence of functional SGLT2, less than 10% of glucose is absorbed through SGLT1; therefore, it is expected that SGLT2 inhibitor therapy would lead to around 90% reduction in T_m but the observed decrease of only 30–50% in T_m can be explained by higher amount of SGLT1-mediated glucose reabsorption [70].

Compound	Preparation strength available	SGLT2/SGLT1 selectivity	Reference
Dapagliflozin	5, 10 mg	1200	[60]
Canagliflozin	100, 300 mg	200	[61]
Empagliflozin	10, 25 mg	2500	[62]
Ertugliflozin	5, 15 mg	2000	[63]
Ipragliflozin	25, 50 mg	254	[64]
Luseogliflozin	2.5, 5 mg	1765	[65]
Tofogliflozin	20 mg	2900	[66]

Table 2.
Preparation strength and SGLT2 versus SGLT1 selectivity of various approved SGLT2 inhibitors.

During preclinical studies with animal models of diabetes as well as in clinical studies with both T2DM and T1DM patients, it has been demonstrated that prolonged SGLT2-I therapy decreased fasting and prandial plasma glucose levels, reduced HbA1C, and improved oral glucose tolerance. They also exerted nephroprotective effects, reduced blood pressure, and increased utilization of fatty acid substrates; thus, they also conferred metabolic benefits [68–73].

SGLT2-I have insulin-independent mechanism of action. They can be used both as monotherapy as well as in combination with other OAHs [74, 75]. Clinical trials have shown that SGLT2-I are effective when administered in combination with metformin [74, 76–78], metformin plus sulfonylurea [79], insulin [80], DPP4 inhibitors [77], and thiazolidinediones [75].

SGLT2-I do not increase the risk of hypoglycemia like other OAH agents. The filtered renal glucose load is directly correlated with plasma glucose level, and there is compensatory increase in SGLT1-mediated glucose reabsorption when SGLT2 is blocked [81]. The other mechanism preventing hypoglycemia is the glucagon secretion from pancreatic alpha cells due to SGLT2 inhibition [31].

SGLT2-I therapy in patients with T2DM increases both plasma glucagon and endogenous glucose production. Despite such physiological changes, patients on SGLT2-I have lower plasma glucose levels than those receiving placebo, possibly because of increased glycosuria and improved insulin sensitivity [82, 83].

SGLT2-I administration changes body metabolism and shifts it to enhanced usage of fat for metabolic needs; consequently beta-hydroxybutyrate levels in plasma increase [69]. The metabolic inflexibility characteristically seen in patients with T2DM and nondiabetic insulin-resistant subjects is an inability to switch from predominantly fatty acid oxidation during fasting state to predominantly glucose oxidation in fed state [84]. Lack of variability in measured respiratory quotient (RQ) between fasting and fed states has been observed as an evidence of metabolic inflexibility. SGLT2-I reduce whole body fasting RQ which is indicative of increased oxidation of fatty acids and amino acids, suggesting its partial restoration [85].

As a consequence of improved glucose homoeostasis, SGLT2-I may slow down glucotoxicity-mediated degeneration of beta cells and thus may also slow down the progression of T2DM. Experimental diabetic animal models on SGLT2-I have shown an improvement in both beta cell mass and functions [86]. Improvement in insulin sensitivity is another beneficial aspect [82, 83].

6.2 Effects on blood pressure

Increased urinary excretion of glucose and sodium leads to mild diuresis coupled with urinary sodium loss. Reduction of extracellular volume occurs which has a favorable effect on blood pressure, and the magnitude of the effect is most apparent in patients with preexisting hypertension [87].

Empagliflozin causes a reduction in systolic blood pressure (SBP) of 4–6 mmHg, whereas diastolic blood pressure (DBP) was similar to placebo with no increase in the heart rate [88]. Similarly, canagliflozin at 300 mg/day resulted in a reduction in SBP of 5.1 mmHg [89].

In analysis of 12 studies with dapagliflozin at 10 mg/day, reduction of 4.4 mmHg and 0.5 mmHg in SBP and DBP, respectively, was seen with no increase in the heart rate compared to placebo [90].

6.3 Effects on body weight

On SGLT2-I therapy, initial weight loss could result from osmotic diuresis. However, sustained weight loss over the period is a consequence of renal glucose

excretion leading to a caloric deficit of about 280 calories/day. This translates into decrease in body weight by 1–3 kg along with decreased visceral adiposity.

In the EMPA-REG trial, empagliflozin resulted in weight loss of about 2 kg [91]. The average weight loss achieved with dapagliflozin and canagliflozin stands between 1 and 3%, while other studies report a loss more than 5% [92, 93].

6.4 Effects on plasma lipids and plasma uric acid

SGLT2-I therapy has been associated with alteration in serum lipid profile. Small increase in low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL) has been observed [76, 78, 94].

Any increase in serum cholesterol is considered to be a risk factor for the development of heart failure and CVDs. However, it may not be relevant clinically, as empagliflozin has been shown to have a protective effect on MACE in EMPA-REG trial [91].

They also promote uric acid excretion and reduce uric acid level in the blood [94] which may contribute to their protective effect on the development of diabetic nephropathy observed in the EMPA-REG renal study [73, 80, 95].

6.5.1 Effects on cardiovascular disease

In EMPA-REG trial, empagliflozin has shown a lower risk for MACE as well as for cardiovascular death and has been found to be protective [88].

In CANVAS Program, canagliflozin has been found to reduce the risk of cardiovascular death or hospitalization for heart failure in patient with a history of T2DM and high CVD risk [96].

Lately, DECLARE-TIMI 58 trial demonstrated the CV safety of dapagliflozin in patients with T2DM who had or were at risk of CVDs. It was found to reduce hospitalization for heart failure as has been seen with other SGLT2-I [97].

Both empagliflozin and canagliflozin has got FDA approval for cardiovascular risk reduction in T2DM patients who are at high risk for such events. The mechanism of cardiovascular protection exerted by SGLT2-I is unknown and seems to be glucose independent. Apart from improved glycemic control, the pleiotropic effects of SGLT2 inhibitors which include their ability to lower blood pressure, reduction in intraglomerular pressure and albuminuria, and amelioration of volume overload are all plausible protective mechanisms.

6.5.2 Effects on liver disease

T2DM affects metabolism in the liver and may manifest as hepatic steatosis and nonalcoholic steatohepatitis (NASH). NASH increases the risk of hepatocellular carcinoma and may result in liver cirrhosis also.

In preclinical studies on rodent models, SGLT2-I have been found to ameliorate nonalcoholic fatty liver disease (NAFLD) and NASH [70–72]. Although SGLT2-I are metabolized by the liver, studies involving patient with mild or moderate hepatic impairment showed dapagliflozin and empagliflozin were well tolerated and required no dose adjustments [98, 99].

Canagliflozin has shown improvement in serum aminotransferases and gamma-glutamyl transferase levels in patients with T2DM [100]. In humans, ipragliflozin has shown reduction in liver fat in patients with T2DM and NAFLD [101]. Empagliflozin addition to the standard treatment of T2DM and NAFLD significantly reduced liver fat, and improved ALT levels were seen [102].

6.5.3 Effects on kidney disease

The kidney is the main target of action for SGLT2-I. Findings from animal models suggest it to be protective against development of diabetic nephropathy which exceeds the nephroprotective effect achieved secondary to improved glycemic control. During early stage of nephropathy, empagliflozin has been shown to prevent glomerular hyperfiltration, attenuate diabetes-associated renal growth, improve expression of inflammation markers, and reduce albuminuria in animal models [24].

Progression of nephropathy was slowed by luseogliflozin in T2DN rats, a genetic model of T2DM associated with severe nephropathy. It also prevents GFR decline and attenuates focal glomerulosclerosis, tubular necrosis, tubulointerstitial fibrosis, and progressive proteinuria [103].

Reduction in estimated GFR and nephroprotective effects has been reported with SGLT2-I in patients with DM [73, 80, 104]. Empagliflozin has been found to attenuate glomerular hyperfiltration in humans. It also reduces micro- and macroalbuminuria, and the effects are independent of the improved glycemic effect [105]. In CANVAS trial, canagliflozin has been found to reduce albuminuria and the albumin-to-creatinine ratio when compared with placebo [106].

Results from a recent meta-analysis have indicated that SGLT2 inhibition preserves renal function in patient with or without renal impairment. They slow down the progression of albuminuria and reduce urinary albumin-to-creatinine ratio in addition to reducing the risk of doubling of the serum creatinine level, initiation of kidney transplant, and death from kidney disease in patient with T2DM with or without history of renal impairment [107].

6.6 Risks for adverse drug effects and restrictions of application

6.6.1 Genital and urinary infections

Increased risk of GMI and urinary tract infections (UTI) are seen in DM due to hyperglycemia and glucosuria. SGLT2-I therapy has been associated with increased risks for GMIs and UTIs. Women otherwise also are more commonly affected than men, and SGLT2-I therapy further increases the risk in them.

When treated with canagliflozin for 4 months, around 10.4% of women and 4.2% of men developed GMIs compared to 3.2% of women and 0.6% of men treated with placebo [108]. Most of the cases were of mild to moderate severity and could be treated with standard antifungal agents successfully.

Canagliflozin therapy in T2DM has been associated with UTIs in 8.7% of women compared to 7.7% treated with placebo. Figures for men were 1.4% versus 0.6%, respectively [109]. Similar findings were reported from pooled analysis of four studies (n = 2477) using empagliflozin. It was concluded that GMI were more common with empagliflozin than placebo (approximately 4 versus 1%, respectively); however, the frequency of UTI was about 8–9% for each [110].

Safety data from meta-analysis of eight studies using canagliflozin and dapagliflozin found that UTIs were more common with SGLT2-I as compared to other OAH (odds ratio, 1.42 [95% CI 1.06, 1.90]) as were GMI (odds ratio, 5.06 [95% CI 3.44, 7.45]) [111].

These studies suggests that most UTIs were mild to moderate, responded well to standard antimicrobial therapy, and rarely led to SGLT2-I discontinuation [112].

6.6.2 Euglycemic ketoacidosis

SGLT2-I therapy has been observed to be associated with small number of cases with EKA during treatment of T1DM and insulin-deficient T2DM [113].

In CANVAS study, the incidence rates were 0.5 per 1000 patient years with canagliflozin 100 mg, 0.8 per 1000 patient years with canagliflozin 300 mg, and 0.2 per 1000 patient years with comparator [114]. In the EMPA-REG trial, the incidence rates were 0.5 and 0.2 per 1000 patient years with empagliflozin 10 and 25 mg, respectively, and 1.2 per 1000 patient years in placebo group [88]. In DECLARE-TIMI 58, using dapagliflozin, the corresponding rates were 0.3%, whereas 0.1% occurred among placebo-treated group [97].

Majority of the cases have been reported from clinical practice rather than trials and have occurred in patients on exogenous insulin. Reduction in insulin dose on starting SGLT2-I has been observed in them. Usually they present with classical diabetic ketoacidosis (DKA) features. However, some cases may present atypically with lower-than-expected hyperglycemia, and it can go unrecognized.

Almost all cases occurred in patients challenged with metabolically stressful events and common precipitants such as surgery, myocardial infarction, stroke, extensive exercise, severe infections, and prolonged fasting.

Low serum bicarbonate and positive urinary ketones may be suggestive but may be inaccurate; therefore, direct measurement of serum betahydroxybutyrate level to confirm the diagnosis of EKA has been recommended by the AACE. Once the diagnosis of EKA is confirmed, SGLT2-I should be discontinued, and DKA protocol should be followed [115].

The increased risk of EKA associated with SGLT2-I therapy may be explained by absolute or relative insulin deficiency, increased glucagon secretion, and stimulation of lipolysis and ketogenesis; however, other ketogenic factors are also involved [31, 69].

Most cases of EKA have occurred in patients with T1DM, which is an off-label use of these agents that is not an FDA-approved indication. Because insulin deficiency may be the most important contributing factor, the AACE recommends against stopping insulin or decreasing the dose excessively. The risk of EKA has recently been shown in the EASE Trial to be dose dependent as lower doses of empagliflozin 2.5 mg were shown to be associated with lower rates of DKA, compared to 10 and 25 mg, respectively [116].

Although not approved for treatment of T1DM and SGLT2-I use is still off-label in T1DM, the AACE encourages clinical trials due to their promising effect on glycemic control in this population [115].

6.6.3 SGLT2 inhibitors and risk of limb amputations

Canagliflozin significantly reduced the risk of CV events by 14% but increased the risk of lower limb amputation in patients with T2DM and high CVD risk (hazard ratio 1.97) versus placebo as seen in CANVAS trial [114].

In the EMAP REG trial using empagliflozin, in T2DM patient with established CVD, the rate of lower limb amputation was similar to placebo group [88].

Recently in DECLARE-TIMI 58 trial, the rate of amputation was similar between the dapagliflozin- and placebo-treated patient (hazard ratio 1.09) [97].

In a recent report, canagliflozin, but not dapagliflozin or empagliflozin, was associated with a higher risk of amputation in a pharmacovigilance analysis using the US FDA Adverse Event Reporting System [117].

Presently the evidence may not be enough to explain a precise causal relationship between canagliflozin and amputation. Neither the underlying mechanisms are currently known, nor do we know whether it is specifically related to canagliflozin. As amputation carries a negative impact on patient's clinical course, understanding predisposing factors and mechanisms of amputation will be crucial to maximize the benefits of SGLT2 inhibitors in clinical practice [117].

6.6.4 Effect on bone health

In CANVAS study, patients treated with canagliflozin had about six additional cases of bone fracture compared to those receiving placebo. However, such an effect could not be replicated in other trials of canagliflozin [118].

Canagliflozin is associated with a small but statistically significant decrease in total hip bone mineral density (BMD) but no statistically significant change in BMD at other sites and without any meaningful changes in most biomarkers of bone turnover [118].

No significant changes in bone density or increase in rate of fracture were observed with dapagliflozin in patients with DM and normal or mildly impaired renal function, but more fractures were observed in dapagliflozin-treated patients with moderate renal impairment (eGFR ≤30–60 mL/min/1.73 m^2) [104]. Empagliflozin however did not show any clear evidence of increase fracture rates in people with T2DM [119].

Furthermore, the absence of SGLT2 in bone or bone marrow makes direct cause-effect hypothesis unlikely. It is known that SGLT2-I induce osmotic diuresis leading to volume depletion which may increase the susceptibility to falls. An increase in fall-related fractures cannot be ruled out as a possible explanation. The exact reason and mechanism are unknown at this time and may possibly be related to factors extrinsic to bone health [28].

Pending further evidence, the US FDA has revised the label of canagliflozin with new warning in September 2015.

6.4.5 Restrictions of application

SGLT2-I are prescribed as once daily oral pill due to their long elimination half-life. They are metabolized in the liver, and inactive metabolites are formed mainly due to glucuronidation.

They are also eliminated partially by renal excretion of parent drugs. Thus, dose adjustment is needed in patients with hepatic and/or renal disorders. They are contraindicated in severe chronic kidney disease.

7. Conclusion

SGLT2-I are a unique emerging class of OAH agents that has addressed fundamental aspects of the unmet needs that challenge physicians treating T2DM patients, such as increased risk of hypoglycemia and weight gain that are usually noticed with other agents such as insulin. On the contrary, SGLT2-I therapy in T2DM is associated with very low risk of hypoglycemia and also promotes weight loss. Moreover, SGLT2-I have been shown to reduce the risk of MACE, all-cause mortality, and hospitalization for heart failure. They have additional renal protective properties besides reducing SBP. In fact, their mode of action through prevention of glucose reabsorption in the kidney makes them work independently from the pancreas, bypassing the problem of progressive beta cell failure that happens in most patients with T2DM over time, and this gives them longer durability. They can be used regardless of duration of disease and can be used as monotherapy as well as in combination as they have been shown to complement actions of other OAH agents including insulin. Considering their unique mechanism of action, they may be useful in impaired glucose tolerance and prediabetes also. The major side effects drawbacks of SGLT2-I is the increased rate of GMI. Another important side effect of SGLT2-I is EKA in T2DM patients during stress and following surgery as

well as in T1DM, which is an off-label use of these medications and seems to be dose dependent. Future drug developments should focus on finding the least effective dose with the least side effects.

Conflict of interest

Authors declare that there is no conflict of interest. No fund or grant was received in any form for this work.

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