Overview on Sodium Glucose Transport Inhibitors as a Therapeutic Tool Against Diabetes Mellitus

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Abstract: In the search for potential new drug targets for the treatment of diabetes, sodium-glucose co-transporters (SGLTs), in particular SGLT2, have been the subject of particular attention. SGLT2 plays an important role in glucose reabsorption in the kidney and SGLT2 inhibitors enhance renal glucose excretion and consequently lower plasma glucose levels. The main target is thereby the early proximal tubule where secondary active transport of the sugar is mediated by the sodium glucose co-transporter SGLT2. The principle behind SGLT2 inhibition involves the improvement of diabetic conditions without increasing body weight or the risk of hypoglycemia. SGLT2 expressed in the proximal renal tubules accounts for about 90% of the reabsorption of glucose from tubular fluid. Genetic defects of SGLT2 result in a benign familial renal glucosuria. Pharmacological agents that block SGLT2 are being tested as potential treatment for type 2 diabetes mellitus.

Key words: Sodium Glucose Transport (SGLT) • Diabetes • Glucosuria • Phlorizin • Dapagliflozin

INTRODUCTION

Sodium-dependent glucose co-transporters (SGLT) are a family of glucose transporter found in the intestinal mucosa (enterocytes) of the small intestine (SGLT1) and the proximal tube of the nephron (SGLT2 in PCT and SGLT1 in PST). They contribute to renal glucose reabsorption. In the kidneys, 100% of the filtered glucose in the glomerulus has to be reabsorbed along the nephron (98% in PCT, via SGLT2). In case of too high plasma glucose concentration (hyperglycemia), glucose is excreted in urine (glucosuria); because SGLT are saturated with the filtered monosaccharide. One must know that glucose is never secreted by the nephron. Diabetes mellitus is the most common metabolic disorder characterized by hyperglycaemia. It is associated with long term microvascular and neurological complications affecting kidney, heart, eyes and nerves [1-6]. Insulin regulates carbohydrate metabolism by aiding the transport of glucose and amino acid from the blood stream into the storage organs such as liver and muscles. In diabetes mellitus, there is hindrance of glucose transport of such a degree that threatens or impairs health [7].

Management of type 2 diabetes mellitus (T2DM) remains complex and challenging. Although a wide range of pharmacotherapy for T2DM is available, including metformin, insulin secretagogues (predominantly sulfonylureas), thiazolidinediones, α-glucosidase inhibitors, insulin and more recently glucagon like peptide-1 agonists and dipeptidyl-peptidase-IV inhibitors, many patients do not achieve glycemic targets, partly due to limiting side effects of current therapies, including weight gain, hypoglycemia, fluid retention and gastrointestinal side effects. Hence, the search for new treatment strategies is ongoing [8].

Among the new therapies on the horizon, sodium-glucose co-transporter 2 (SGLT2) inhibitors seem promising and there are a number of ongoing phase II and III clinical trials with a variety of these compounds. SGLT2 is almost exclusively expressed in the renal proximal tubules and accounts for 90% of the renal glucose reabsorption [8]. SGLT2 inhibitors work independently of insulin and lead to negative energy balance by enhanced urinary glucose excretion. This makes it mechanistically possible for this class of drugs to reduce glucose levels without causing hypoglycemia and weight gain. However, the side effect profile remains to be further elucidated in ongoing phase III trials and these compounds will need to be proven safe from a renal and cardiovascular perspective to meet current regulatory requirements for new diabetes treatment.
Table 1: SGLT 1 and SGLT 2 and their properties

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Acronym</th>
<th>Tissue distribution in proximal tubule</th>
<th>Na⁺: Glucose Co-transport ratio</th>
<th>Contribution to glucose reabsorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC5A1</td>
<td>Sodium/GLucose coTransporter 1</td>
<td>SGLT1</td>
<td>S3 segment</td>
<td>2:1</td>
<td>10</td>
</tr>
<tr>
<td>SLC5A2</td>
<td>Sodium/GLucose coTransporter 2</td>
<td>SGLT2</td>
<td>predominately in the S1 and S2 segments</td>
<td>1:1</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 2: Types of Sodium glucose transporters

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Substrate</th>
<th>Tissue Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC5A1</td>
<td>SGLT1</td>
<td>Glucose and galactose</td>
<td>Small intestine, trachea and kidney</td>
</tr>
<tr>
<td>SLC5A2</td>
<td>SGLT2</td>
<td>Glucose</td>
<td>Kidney</td>
</tr>
<tr>
<td>SLC5A4</td>
<td>SGLT3</td>
<td>Glucose sensor</td>
<td>Small intestine, lung, uterus, thyroid and testes</td>
</tr>
<tr>
<td>SLC5A9</td>
<td>SGLT4</td>
<td>Mannose, glucose, fructose and 1,5-AG</td>
<td>Small intestine, kidney, lung and liver</td>
</tr>
<tr>
<td>SLC5A10</td>
<td>SGLT5</td>
<td>Glucose and galactose</td>
<td>Kidney</td>
</tr>
<tr>
<td>SLC5A11</td>
<td>SGLT6</td>
<td>Myo-inositol, xylose and chiro-inositol</td>
<td>Pinal cord, kidney, brain and small intestine</td>
</tr>
</tbody>
</table>

Types of SGLT: The two most well known members of SGLT family are SGLT1 and SGLT2 (Tables 1, 2), which are members of the SLC5A gene family [9, 10].

Including SGLT1 and SGLT2, there are total seven isoforms in the human protein family SLC5A, several of which may also be sodium-glucose transporters [11, 12].

Mechanism of SGLT: First, the Na⁺/K⁺ ATPase pump on the basolateral membrane of the proximal tubule, cell actively (requires ATP) transports sodium from this cell into the peritubular capillary. This creates a downhill sodium gradient inside the proximal tubule cell. The SGLT proteins use the energy from this downhill sodium gradient created by the ATPase pump to transport glucose across the apical membrane against an uphill glucose gradient. Therefore, these co-transporters are an example of secondary active transport (The GLUT uniporters then transport the glucose across the basolateral membrane, into the peritubular capillaries). Both SGLT1 and SGLT2 are known as symporters, since both sodium and glucose are transported in the same direction across the membrane.

Glucose Transport Across Biological Membranes: Glucose absorption at the enterocytes, reabsorption at the renal tubules, transport across the blood-brain barrier and uptake and release by all cells in the body are affected by two groups of transporters: glucose transporters (GLUTs) and sodium-glucose co-transporters (SGLTs). GLUTs are facilitative or passive transporters that work along the glucose gradient. They belong to the SLC2 (solute carrier family 2) gene family, which has 13 members: GLUT 1-12 and the H⁺-myoinositol co-transporters. They are expressed in every cell of the body [13, 14].

SGLTs co-transport sodium and glucose into cells using the sodium gradient produced by sodium/potassium ATPase pumps at the basolateral cell membranes. They belong to the SLC5 gene family, which has nine members with known functions. Of these, six are plasma membrane sodium/substrate co-transporters for solutes such as glucose, myoinositol and iodide (SGLT 1-6). SGLT1 is primarily expressed in the small intestine but is also found in the trachea, kidney and heart and its predominant substrates are glucose and galactose. SGLT2 is expressed in the S1 and S2 segments of the proximal convoluted tubules and is responsible for renal reabsorption of glucose [15]. SGLT2 RNA is minimally expressed in the ileum, the level of which was insignificant by Northern blot analysis [16]. Although one study using real-time PCR suggests widespread SGLT2 RNA expression in a variety of tissues [17], there is no published data to show that SGLT2 protein is found outside the kidney. There have been attempts to examine expression of SGLT proteins, but availability of antibodies that are specific enough seems to be the limiting factor. Therefore, the functional significance of mRNA expression in nonrenal tissues has not been established. However, there is some evidence for SGLT2 mRNA expression by real-time PCR as well as for SGLT2 protein expression in the placenta by Western blot analysis [18]. SGLT1 gene mutations lead to glucose-galactose malabsorption, which causes potentially fatal diarrhea. The oral rehydration therapy that saves millions of lives from infectious diarrhea works via SGLT1 transporters in the enterocytes [19, 20].

Much of the evidences for SGLT2 being a major pathway for renal glucose reabsorption comes from genetic studies of individuals with familial renal glucosuria [21, 22]. Mutations in the SLC5A2 gene encoding SGLT2 lead to familial renal glucosuria, which is inherited as an autosomal recessive trait and is characterized by normal blood glucose levels, normal oral glucose tolerance test results and isolated persistent glucosuria [23, 24]. A study analyzing 23 families with
index cases of renal glucosuria found that each family had a unique mutation in the SGLT2 gene. Individuals who were found to be carriers of two mutated alleles showed severe glucosuria, defined by a urinary glucose of more than 10 g/1.73 m² per 24 hrs (55 mmol/1.73 m² per 24 hrs). The index patients who presented with mild glucosuria and the family members of cases of severe glucosuria were shown to be heterozygous carriers of SGLT2 mutations [22, 25]. Another study found 20 different SLC5A2 mutations within 17 pedigrees. Glucosuria was mild in heterozygotes ranging from 2.7 to 10 g/1.73 m² per 24 hrs, whereas it was severe in homozygotes ranging from 15.2 to 86.5 g/1.73 m²/24 hrs. Two patients in the homozygous group had plasma renin and serum aldosterone concentration raised to an average of 4.6- and 3.1-fold, respectively, suggesting activation of compensatory mechanisms [26].

Renal Glucose Transporter in Normal: Kidneys play a very important role in glucose homeostasis. Blood glucose is freely filtered by the glomeruli and is essentially completely reabsorbed from the proximal tubules via sodium-coupled transporters in the brush border membrane. The glomeruli filter about 144 g of glucose per 24 hrs, nearly 100% of which is reabsorbed in the renal tubules. When blood glucose levels reach the renal threshold for reabsorption, which is about 8 to 10 mmol/liter (180 mg/dl), glucosuria starts to develop [27]. The proximal tubule has traditionally been divided into S1, S2 and S3 segments based on the cell morphologies, although more recent ultra structural analyses of computer-assisted three-dimensional reconstruction of mouse proximal tubules revealed no obvious morphological segmentation of the proximal tubule [28]. There is evidence, however, for heterogeneity of sodium-dependent glucose transport along the proximal tubule. The S1 and S2 segments of the proximal convoluted tubules show low affinity and high capacity for sodium-dependent glucose absorption, whereas the more distal parts show higher affinity and low capacity for the same [29]. SGLT2 is located in the S1 and S2 segments where the majority of filtered glucose is absorbed and SGLT1 is located in S3 segments responsible for reabsorbing the remaining glucose [15, 30].

Renal Glucose Transporter in Diabetics: Renal tubular reabsorption is known to undergo adaptations in uncontrolled diabetes; particularly relevant in this context is the up-regulation of renal GLUTs. The increase in extracellular glucose concentration in diabetes lowers its outwardly directed gradient from the tubular cells into the interstitium. Hence, up-regulation of SGLT2 is an important adaptation in diabetes to maintain renal tubular glucose reabsorption. SGLT2 mRNA expression is up-regulated in diabetic rat kidneys and this up-regulation is reversed by lowering blood glucose levels [31]. Human exfoliated proximal tubular epithelial cells from fresh urine of diabetic patients express significantly more SGLT2 and GLUT2 than cells from healthy individuals [32]. There is also evidence for up-regulation of GLUT2 gene expression in renal proximal tubules in diabetic rat models [33-35]. Uncontrolled diabetes leading to increased expression of SGLT2 has practical significance in that the inhibitors are likely to produce greater degrees of glucosuria in the presence of higher prevailing plasma glucose levels. This has been shown in preclinical studies with the nonspecific SGLT inhibitor, T-1095 [36]. Interestingly, this up-regulation of SGLT2 receptors is also seen in renovascular hypertensive rat models. The authors speculated that angiotensin II-induced SGLT2 over expression probably contributes to increased absorption of Na+ and thereby development or maintenance of hypertension. Rats treated with either Ramipril or Losartan showed significant reduction in the intensity of immunostaining and levels of SGLT2 protein and mRNA. This may have relevance in diabetes, given the high prevalence of hypertension in diabetes [37].

Discovery of Therapeutic Potential of SLGTs to Produce Glucosuria: Phlorizin is a glucoside consisting of a glucose moiety and two aromatic rings (aglycone moiety) joined by an alkyl spacer. In the 19th century, French chemists isolated it from the bark of apple tree to be used in treatment of fever and infectious diseases, particularly malaria. Von Mering observed in 1886 that Phlorizin produces glucosuria. It has been used as a tool for physiological research for more than 150 yr [38]. In 1975, DeFronzo et al. showed that infusion of Phlorizin in dogs increased fractional excretion of glucose by 60%, whereas glomerular filtration rate and renal plasma flow remained unchanged [39]. Phlorizin is a high-affinity competitive inhibitor of Na-dependent glucose transport in renal and intestinal epithelia [40]. Hence, it causes malabsorption of glucose and galactose from the small intestines and of glucose from the renal tubules. Phlorizin caused heavy glucosuria and marked inhibition of glucose uptake in the small intestine during enteric perfusion in normal rats. It also significantly reduces blood glucose on oral glucose tolerance test in mice and lowers blood glucose in
streptozotocin-induced diabetic rats [41]. It improves counter-regulatory responses reducing the risk of hypoglycemia in animal models [42]. In 1986, Unger’s group reported that i.v. glucose failed to suppress the marked hyperglucagonemia found in insulin-deprived alloxan-induced diabetic dogs; however, when hyperglucagonemia was corrected by phlorizin, the hyperglucagonemia became readily suppressible. Phlorizin treatment of partially pancreatectomized rats completely normalized insulin sensitivity but had no effect on insulin action in controls [43, 44], suggesting that the effect on insulin sensitivity was by reversal of glucotoxicity, rather than by a direct effect on insulin sensitivity. Animal studies with Phlorizin have shown that its effect of changing the ambient glucose independent of insulin levels can up-regulate the glucose transport response to insulin in adipose cells, which may be as a result of changes in GLUT functional activity [45].

These findings provided important proof of concept data, although Phlorizin itself is unsuitable for development as a drug for the treatment of diabetes because of its non-selectivity and low oral bioavailability [36, 46]. T-1095 is a synthetic Phlorizin derivative, which unlike Phlorizin is absorbed into the circulation on oral administration and is metabolized to its active form T-1095A. It suppresses the activity of SGLT1 and -2 in the kidney and increases urinary glucose excretion in diabetic animals, thereby decreasing blood glucose levels. With long-term T-1095 treatment, both blood glucose and glycosylated hemoglobin (HbA1c) levels were reduced in streptozotocin-induced diabetic rats and the obese insulin resistant yellow KK rat models [36]. Chronic administration of T-1095 lowered blood glucose and HbA1c levels, partially improved glucose intolerance and insulin resistance and prevented the development of diabetic neuropathy in the diabetic insulin-resistant GK rat models. There were no adverse side effects reported at the end of the study. This drug, however, did not proceed to clinical development, presumably because it also inhibits SGLT1 [47].

SLGT Inhibitors in Clinical Development

Dapagliflozin: Dapagliflozin is a potent and highly selective SGLT2 inhibitor. The elimination half-life after intraarterial administration was 4.6, 7.4 and 3.0 hrs in rats, dogs and monkeys, respectively [48]. Single and multiple ascending dose (SAD and MAD) studies with dapagliflozin confirmed that it has a pharmacokinetic profile consistent with once-daily dosing and produces a dose-dependent increase in glucosuria in humans. Dapagliflozin was rapidly absorbed after oral administration and maximum plasma concentrations (C_{max}) were observed within 2 hrs of administration. The mean half-life after the last dose in the MAD study ranged from 11.2 to 16.6 hrs and the data were similar for the SAD study with high dose. In the MAD study, a dose of 100 mg produced urine glucose of 58.3 g per 24 hrs and 55.4 g per 24 hrs on d 14. Dapagliflozin had no effect on urine and serum electrolytes, serum albumin, osmolality, or renal tubular markers such as N-acetyl-b-d-glucosaminidase and β2-microglobulin. Two events of mild asymptomatic hypoglycemia were reported in the SAD study. No treatment-related serious adverse events were reported in either study [49]. In a phase IIa study, 47 patients with T2DM were randomized to receive 5, 25, or 100 mg of dapagliflozin or placebo for 14 d. Those receiving 25 and 100 mg dapagliflozin had approximately 40% inhibition of renal glucose reabsorption as compared with baseline resulting in glucose excretion of up to 70 g per 24 hrs. Two of the 24 women who received dapagliflozin were diagnosed with mild vulvovaginal mycotic infections that resolved in 4 d with treatment. The most frequently reported treatment emergent adverse events were gastrointestinal, including constipation, nausea and diarrhea. There were no drug discontinuations due to adverse events [50]. A phase IIb multiple-dose study to evaluate safety and efficacy of dapagliflozin-randomized T2DM patients to five dapagliflozin doses (2.5, 5, 10, 20, or 50 mg), metformin extended release, or placebo for 12 wk has recently been reported. Dapagliflozin improved hyperglycemia and caused weight loss by inducing controlled glucosuria with urinary loss of approximately 200-300 kcal/d. There was a weight loss of 2.5 to 3.4 kg in the dapagliflozin-treated patients, compared with 1.2 kg with placebo and 1.7 kg with metformin. Dapagliflozin treatment was not associated with clinically significant osmolality, volume, or renal status changes. There was also no compensatory increase in hunger as assessed by visual analog scales. The rates of bacterial urinary tract infections (UTIs) were similar in the treatment and placebo arms, but there was higher incidence of genital infections in the dapagliflozin vs. placebo, especially at higher doses [51].

Sergliflozin: Sergliflozin (KGT-1251), a prodrug of SGLT2 inhibitor Sergliflozin A was developed by Kissel Pharmaceuticals, Japan and currently, it is being developed by GlaxoSmithKline. It has been shown 7-fold selectivity for human SGLT2 Vs human SGLT1 in cell culture system. It has been induced glucosuria in healthy
mice, rats and dogs and also lower postprandial blood glucose in diabetic rats independently of insulin secretion [52]. According to study conducted by Hussey et al., sergliflozin has shown dose dependent glucosuric effect [53]. The study was conducted in 18 healthy over weight and obese subjects (18-55 years) to evaluate safety, pharmacokinetic and pharmacodynamic of sergliflozin over 14 days of dosing. These eighteen subjects were divided in two cohorts of equal subjects and six subjects of each cohort were kept on 500 and 1000 mg of sergliflozin and rest of three subjects in each cohort were on placebo. The treatment was given three times per day for 14 days. Sergliflozin was well tolerated in both cohorts and had stable pharmacokinetic parameters. After 14 days treatment, subjects treated with sergliflozin reduced bodyweight of average 1.5kg compare to placebo treated subjects. Hypoglycemia was not reported. The urinary electrolyte level was raised on day 1 in sergliflozin treated subjects but it was resolved by day 14. Two randomized, double blinded, placebo controlled and single dose escalation crossover studies were done to evaluate safety, pharmacokinetic and pharmacodynamic of sergliflozin. In one study sergliflozin was given in a dose range of 5-500 mg in 14 healthy subjects while in other study dapagliflozin was given in dose range of 50-500 mg in 8 type 2 diabetes patients. No variation was observed in pharmacokinetic parameters between two groups and sergliflozin has shown dose dependent glucose excretion in urine. The duration of glucose excretion was related to plasma concentration of sergliflozin A. In subjects with type 2 diabetes, 500 mg segliflozin reduced mean plasma glucose from 18.2 mmol to 11.2 mmol/L, with minor, transient alterations in urine electrolytes. Some minor adverse events like headache, sore throat in healthy subjects and headache, dyspepsia in diabetic patients were seen. Thus, sergliflozin have shown promising profile for treatment of diabetes along with obesity and also excellent safety.

Remogliflozin Etabonate: Remogliflozin etabonate is metabolized to its active form remogliflozin, which is a benzylpyrazole glucoside. Its skeleton differs from that of phlorizin, T-1095, or sergliflozin and hence is from a new category of SGLT2 inhibitors. Its inhibitory effect for human SGLT2 is approximately three times greater than that of Phlorizin, but for SGLT1 it was only 1/20 that of Phlorizin in in vitro studies. Animal studies have shown good linearity between urinary glucose excretion and the dose of Remogliflozin etabonate. It did not significantly alter the plasma glucose level in 16-h fasted normal rats [54]. A study with 13 T2DM patients supported its co-administration with metformin in patients with T2DM with minimal risk of hypoglycemia. There was no drug interaction affecting the pharmacokinetics of either drug [55]. In another study, Remogliflozin etabonate administered to 10 healthy subjects (doses ranging from 20 to 1000 mg) and six subjects with T2DM (doses ranging from 50 to 500 mg) was rapidly absorbed and converted to remogliflozin, which had a plasma half-life of 120 min across doses and groups. It caused a dose-dependent increase in urinary glucose excretion in all subjects. There were no effects on plasma electrolytes and no serious adverse events [56]. Remogliflozin etabonate also showed antihyperglycemic effects in both streptozotocin-induced diabetic rats in oral glucose tolerance and in diabetic (db/db) mice in the fed condition. Chronic treatment with Remogliflozin etabonate reduced the levels of fasting plasma glucose and glycated hemoglobin. In high-fat diet-fed Goto-Kakizaki rats, remogliflozin etabonate improved hyperglycemia, hyperinsulinemia, hypertriglyceridemia and insulin resistance. Thus study performed on rodent models by Fujimori et al., suggests that remogliflozin etabonate may be a new and useful drug for the treatment of diabetes.

Merits of SGLT Inhibitors: Weight loss or weight maintenance, is a key target for any type 2 diabetes treatment.

No hypoglycemia because SGLT2 inhibitors do not induce insulin secretion or inhibit hepatic glucose production. Improve insulin sensitivity and indirectly preserve β-cells by depletion of toxic glucose concentration in blood.

SGLT2 inhibitors also produce osmotic diuretic effect which may be advantageous in patients with hypertension and CHF.

Demerits of SGLT Inhibitors: There may be a risk of negative effect of glucosuria on the kidneys, polyuria and increased thirst, but there is no strong evidence about it. Another theoretical problem in relation to the genitourinary tract is increased risk for either bacterial or fungal infection, but only long term clinical trial can answer about this risk [57].

Other SGLT Inhibitors in Clinical Development [58]:
A number of other SGLT2 inhibitors are under development like:
CONCLUSION

SGLT2 inhibitors have a unique mechanism of action and have the potential to become a new treatment for T2DM. Several phase III trials with these compounds are ongoing and if their efficacy and safety profile are proven to be adequate, these agents may gain a place in the management of T2DM, particularly where weight gain is a concern. The potential for use as an insulin-sparing agent in patients on insulin has been highlighted in a recent trial and a future role in the management of type 1 diabetes mellitus cannot be ruled out, although SGLT2 inhibitors have not yet been tested for this indication.

REFERENCES


