Inhibition of renal glucose reabsorption as a novel treatment for diabetes patients

Inibição da reabsorção renal de glicose como uma nova forma de tratamento para pacientes com diabetes

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Abstract

The importance of the kidney in glucose homeostasis has been recognized for many years. Recent observations indicating a greater role of renal glucose metabolism in various physiologic and pathologic conditions have rekindled the interest in renal glucose handling as a potential target for the treatment of diabetes. The enormous capacity of the proximal tubular cells to reabsorb the filtered glucose load entirely, utilizing the sodium-glucose co-transporter system (primarily SGLT-2), became the focus of attention. Original studies conducted in experimental animals with the nonspecific SGLT inhibitor phlorizin showed that hyperglycemia after pancreatectomy decreased as a result of forced glycosuria. Subsequently, several compounds with more selective SGLT-2 inhibition properties ("second-generation") were developed. Some agents made it into pre-clinical and clinical trials and a few have already been approved for commercial use in the treatment of type 2 diabetes. In general, a 6-month period of therapy with SGLT-2 inhibitors is followed by a mean urinary glucose excretion rate of ~80 g/day accompanied by a decline in fasting and postprandial glucose with average decreases in HgA1C ~1.0%. Concomitant body weight loss and a mild but consistent drop in blood pressure also have been reported. In contrast, transient polyuria, thirst with dehydration and occasional hypotension have been described early in the treatment. In addition, a significant increase in the occurrence of uro-genital infections, particularly in women has been documented with the use of SGLT-2 inhibitors. **Conclusion:** Although long-term cardiovascular, renal and bone/mineral effects are unknown SGLT-2 inhibitors,

Resumo

A importância do rim na homeostase de glicose é reconhecida desde há muitos anos. Observações recentes, indicando um papel maior do metabolismo renal da glicose em várias condições fisiológicas e patológicas, reavivaram o interesse no manuseio renal de glicose como um alvo em potencial para o tratamento do diabetes. A enorme capacidade das células tubulares proximais para reabsorver a carga total de glicose filtrada, utilizando o sistema de co-transporte de sódio e glicose (SGLT), tornou-se o foco de atenção. Estudos originais realizados em animais experimentais com o uso do inibidor não-específico da SGLT florizina, demonstraram que a hiperglicemia após pancreatectomia diminuiu como resultado de glicosúria forçada. Posteriormente, foram desenvolvidas diversas substâncias com propriedades mais seletivas de inibição da SGLT-2 ("segunda geração"). Vários agentes foram usados em ensaios pré-clínicos e clínicos, e alguns já foram aprovados para uso comercial no tratamento da diabetes tipo 2. Em geral, os dados clinicos mostram que um período de 6 meses de tratamento com inibidores da SGLT-2 é seguido por uma taxa de excreção de glicose urinária média de ~ 80 g/dia, acompanhado por uma queda na glicemia de jejum e pós-prandial e com redução média na HbA1C de - 1.0%. Também foram relatados perda concomitante no peso corpóreo e uma leve mas consistente queda da pressão arterial. Em contraste, eventos adversos transitórios como poliúria, sede com desidratação e hipotensão ocasional foram descritos na fase inicial de tratamento. Além disso, um aumento significativo na ocorrência de infecções urogenitais, particularmente em mulheres, foi documentado com o uso de inibidores da SGLT-2. Os efeitos cardiovasculares, renais e ósseo/minerais a longo prazo destes agentes ainda são desconhecidos. **Conclusão:** Os inibidores da SGLT-2, se usados de forma criteriosa e em pacientes adequados, representam uma opção terapêutica única, que explora um

if used with caution and in the proper patient provide a unique insulin-independent therapeutic option in the management of obese type 2 diabetes patients.

Keywords: *diabetes mellitus*, type 2/therapy; glycosuria; kidney, sodium-glucose transporter 2.

novo mecanismo de ação anti-hiperglicêmico independente da insulina. Estão portanto indicados, tanto na monoterapia ou em combinação com outros agentes no tratamento de pacientes obesos com diabetes tipo 2.

Palavras-chave: *diabetes mellitus* tipo 2/terapia; glicosúria; rim; transportador 2 de glucose-sódio.

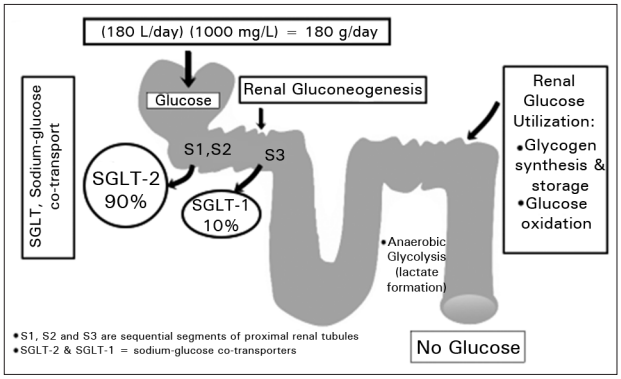
Introduction

The importance of the kidney in glucose homeostasis has been recognized for many years.[1,2] A critical observation indicating that the renal contribution to glucose regulation and counter-regulation might perhaps be of greater significance than previously anticipated was reported in the early 1990's.[3] This publication rekindled the interest in the role of the kidney in glucose metabolism and several studies in animals[4-6] and humans[7-10] subsequently demonstrated the potential impact of renal glucose handling in various physiologic and pathologic conditions. Concomitantly, the pharmacological development of new agents capable of inhibiting renal glucose reabsorption was accelerated and has now reached clinical relevance. As a result, the kidney has become an additional target for anti-diabetic medications.

Renal glucose handling includes free glomerular filtration with complete proximal tubular reabsorption into the renal interstitial fluid space. Renal gluconeogenesis that takes place in the proximal tubular cells adds a small fraction to the glucose load that exchanges with the peri-tubular capillaries along the proximal nephron. In the distal nephron, glucose extracted can be either stored in the form of glycogen or oxidized to generate energy. In turn, no glucose is excreted in the urine and nearly all filtered glucose load perfusing the distal nephron is restored to the peripheral circulation, after mixing in the renal vein with the remainder 80% of unfiltered blood.[11]

The evidence for the heterogeneity of glucose handling within the nephron (Figure 1) is substantiated by numerous reports in the literature.[1,12-15] Several studies have defined the molecular structure and the enormous enzymatic and transport capacity of the renal tubules. The sodium-glucose co-transport system (SGLT) is located at the luminal membrane of the proximal renal tubular cells.[15] These cells contain specific enzymes that enable glucose synthesis *de novo* [renal gluconeogenesis],[1,12] although no enzymatic activity for concomitant glucose utilization, storage or oxidation has ever been identified in proximal tubules.[14]

Figure 1. Schematic representation of the nephron showing the heterogeneity of glucose handling by the kidney. Plasma glucose is freely filtered at the glomerulus and completely reabsorbed in the proximal tubules. The SGLT-2 transporters located at the luminal membrane of cells in the S1 and S2 segments are responsible for 90% of total glucose re-uptake. The SGLT-1 transporters located downstream in the S3 segment of the proximal tubules account for the remainder 10% of the glucose load reabsorbed into the renal interstitial fluid. The process of renal gluconeogenesis occurs exclusively in the proximal tubular cells, whereas glucose utilization is limited to the distal nephron. Glycogen synthesis and storage, as well as complete glucose oxidation are detected only in cells of the distal nephron and, partial oxidation (anaerobic glycolysis) with formation and release of lactate is a characteristic of the hypoxic medullary regions of the kidney.



In contrast, the distal nephron has sufficient biochemical capability to metabolize the glucose extracted from the peri-tubular fluid and, the renal medulla is in fact an obligatory site for glucose oxidation.[13,14] Because most of the energy required by the mega-transport tubular activity is supplied primarily from the oxidation of fatty acids, glucose sparing by the kidney is enabled and represents a critical aspect in the maintenance of whole-body glucose homeostasis.

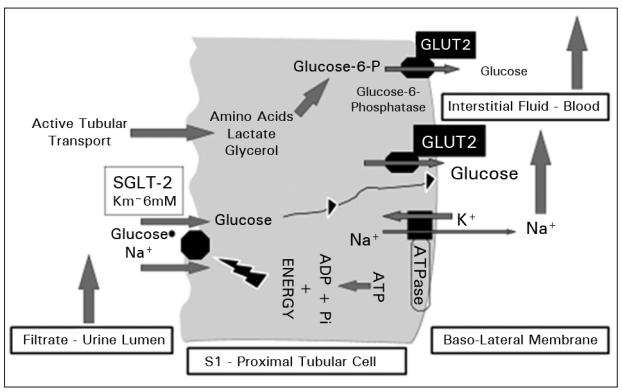
Renal glucose transport and metabolism

Various glucose transmembrane transport systems and intracellular metabolic pathways have been well characterized in the kidney. Most information is derived from *in vitro* cell preparations[16-18] and renal perfusion studies using experimental animal models.[19-21] Analogous

findings have been reported in a few studies conducted in human kidney.[22,23] Glucose is actively transported from the lumen to the tubular cells essentially by two transmembrane proteins: one with high capacity-low affinity termed sodium-glucose transport SGLT-2 and a second with high affinity-low capacity transporter termed SGLT-1. The SGLT-2 is found in the earlier S1 and S2 segments of the proximal tubule and the SGLT-1 in the S3 segment of the proximal tubules,[15,17] which is also abundant in the enterocytes of the intestinal mucosa.[21]

Considering that nearly 180 liters of plasma are filtered daily by the kidneys with plasma glucose concentrations ranging between 80-120 mg/dl (an average of 100 mg/dl), the glucose load that crosses the glomeruli is estimated to be around 180 grams per day (Figure 2). Circulating glucose is neither protein-bound nor attached to macromolecules and thus, is freely filtered at the glomerulus. The ultrafiltrate carries the glucose towards the luminal side of the early S1 segment in the proximal tubules, where SGLT-2 is located. The active process of re-uptake of glucose is coupled with the transport of sodium cations and the complex is transferred to the cell membrane at the level of the S1 segment.

Figure 2. Diagram of a proximal tubular cell located in the S1 segment with transport activity and metabolic pathways leading to effective sodium-glucose reabsorption and gluconeogenesis. Glucose coming from the glomerular filtrate enters the urine lumen, couples with sodium ions and binds avidly to the high capacity-low affinity (Km~6.0 mM) SGLT-2 transporter. The energy required to actively transport the sodium-glucose complex into the cell is supplied by ATP generated by the sodium-potassium ATPase pump action, located in the baso-lateral membrane. Proximal tubules utilize primarily fatty acid oxidation to meet their energy demands and are not capable of metabolizing glucose. Thus, glucose reaches the baso-lateral cell membrane intact, where the GLUT-2 transporter promotes a facilitated passive transport, in favor of a glucose concentration gradient. Gluconeogenesis occurs from precursors such as amino acids, lactate and glycerol arising in the urine lumen via active tubular transport. The presence of the enzyme glucose-6-phosphatase enables de-phosphorylation of newly formed glucose-6-phosphate and glucose is also released into the interstitial fluid, via GLUT-2 facilitated transport. The sodium and glucose-rich proximal interstitial fluid exchanges with blood in the peri-tubular capillaries and perfuses the distal nephron.



This same system is also utilized to carry amino acids from the lumen into the proximal tubular cells. The SGLT-2 transport activity across the luminal membrane is driven by an electro-chemical gradient, which is created by the action of the ATPase-mediated sodium-potassium pump located in the baso-lateral membrane of the cells.[21] The energy consumed in this active transport process is supplied entirely from ATP derived the oxidation of intracellular fatty acids.[11] As glucose builds up inside the proximal tubular cells, a facilitated passive transport in favor of a concentration gradient and mediated by GLUT-2 transporters, transfers intact glucose molecules out and into the surrounding renal interstitial fluid.[24] In addition to restoring glucose (and amino-acids) to the interstitial fluid and eventually to the peripheral circulation, this sodium co-transport process also contributes to the maintenance of fluid and electrolyte balance by the kidney.[15]

In physiologic conditions the high capacity of the SGLT-2 co-transport system is responsible for the reabsorption of nearly 90% of all filtered glucose load. The re-uptake of the remainder 10% of the glucose load is a function of the low-capacity-high affinity SGLT-1 co-transporter and takes place downstream in the S3 segment of the proximal tubules. It is of interest however that despite its minor contribution to glucose re-uptake in the kidney, SGLT-1 represents a major mechanism via which glucose and galactose derived from the meals are absorbed in the intestines.[20,21]

On the other hand, the fact that SGLT-2 is exclusively found in the proximal tubules of the kidney, as opposed to SGLT-1 or GLUT-2, makes this transporter suitable for more specific renal pharmacologic interventions. Thus, the possibility of interfering with the activity of the SGLT-2 has become of considerable clinical significance. It should be emphasized that there is a minor contribution to the glucose load released into the interstitial fluid provided by the process of renal gluconeogenesis.[1,3,6]

This is possible because proximal tubular cells contain the enzymes necessary to synthesize glucose *de novo*, including *glucose-6-phosphatase*, the last step which enables newly-formed glucose to be *de-phosphorylated* and then secreted into the extra-cellular space.[14] The fact that there is no detectable biochemical capacity in the proximal tubular cells to either utilize or store glucose, enables the entire filtered glucose load to be released unaffected

into the renal interstitium. Thus, together with the small fraction of newly-synthesized glucose added by the proximal tubules the glucose-rich interstitial fluid exchanges with the renal venous capillaries and reaches the renal vein.

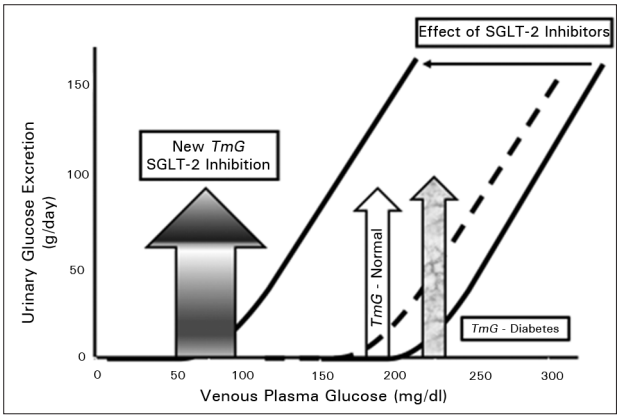
Unlike the proximal nephron, cells in the distal nephron are fully capable of glucose utilization. In physiologic conditions, the amount of glucose utilized in the distal segments of the nephron equals that derived from the *renal gluconeogenesis* in proximal tubules. This is confirmed by the common finding that the arterial-renal vein blood glucose concentration difference in the post-absorptive state is near zero. This is not the case however in more prolonged fasting conditions and during hypoglycemia, when a net contribution of the kidney to systemic glucose appearance has been demonstrated.[7-9,25,26] The glucose extracted in the distal nephron is directed either towards glycogen synthesis for storage or to oxidative pathways, depending upon the local energy demands. Whether this glucose uptake is mediated via an insulin-dependent mechanism or a facilitated transport still remains controversial.[3,7-9]

Renal glycogen accumulation is thought to provide for immediate local energy needs, when blood-borne glucose supply lags behind. The renal oxidation of glucose can be partial (*anaerobic glycolysis)* with the release of lactate and ATP, or complete, a mitochondrial process that yields H_2O, CO_2 and ATP. Partial anaerobic glucose oxidation is more prevalent in hypoxic medullary renal conditions.[12-14] Renal glucose utilization occurs exclusively in distal tubular cells and, in the absence of *glucose-6-phospatase* activity no glucose is released back into the interstitial fluid.[14] Moreover, the small amount of glucose utilized distally does not affect the total glucose load leaving the nephron into the renal veins.

Glucose handling by the kidney

The fate of the glucose filtered, reabsorbed and excreted in the urine in normal and hyperglycemic conditions depends upon the glomerular filtration rate, the prevalent plasma concentration of glucose and the total transport capacity of the proximal tubules (Figure 3). There is a linear relationship between the filtered glucose load at the glomerulus and plasma glucose and thus, the glucose appearance in the ultrafiltrate will be higher or lower as plasma glucose concentration increases or decreases, respectively.

Figure 3. This graph summarizes the urinary glucose excretion and the renal threshold *TmG* values ("splay") calculated in normal healthy individuals (dotted line) and in patients with diabetes prior to and following the administration of SGLT-2 inhibitors (solid lines). The vertical axis expresses urinary glucose excretion rates in grams per day and the horizontal axis represents venous plasma glucose concentrations in mg/dl. Note that in patients with diabetes there is an elevated renal threshold (*TmG*) that is substantially reduced by the effect of SGLT-2 inhibitors.



To a lesser extent, the same is true for bi-directional changes in the glomerular filtration rate. The proximal tubular reabsorption rate, on the other hand is only linear with the glucose load reaching the luminal membrane within a "normal" glycemic range. Once the maximum tubular reabsorption capacity of the kidney (*Tmax*,) is attained the transport process becomes saturated and glucose spills into the urine. It is worth mentioning that since the *Tmax* for glucose varies considerably among the nearly 2 millions nephrons in both kidneys, the maximum transport capacity is actually a "splay" or a range of values estimated to be around the calculated *Tmax*.[21,27,28]

The 'splay" is derived as the rounding of the curves for maximum glucose reabsorption and excretion rates, which show a non-linear transition as the *Tmax* value is approached. The *Tmax* can be determined by artificially elevating plasma glucose levels in a stepwise fashion up to 400-500 mg/dl with simultaneous measurements of the glomerular filtration rate, plasma and urine glucose concentrations, and urine output at given intervals. In individuals with normal kidney function, the calculated maximum tubular glucose transport has been reported between 350-450 mg/min, which corresponds to mean mean venous glucose concentration in the range of 180-200 mg/dl.

Hence, the normal renal threshold (*TmG*) is often referred to as the venous plasma glucose concentration ~180 mg/dl.[27] Once this value is exceeded the SGLT system saturation transport capacity is

passed and glycosuria ensues. *TmG* varies with changes in glomerular filtration rates such that during pregnancy or with a unilateral kidney, when glomerular filtration rates increase, glucosuria will occur at plasma glucose concentrations below 180 mg/dl (lower *TmG*). Conversely, when glomerular filtration decreases such as in chronic kidney disease, glucosuria is seen at plasma glucose levels higher than 220 mg/dl.[27,28] Of additional interest, some apparently healthy individuals inherit a genetic abnormality characterized by a defective SGLT transport system and thus, have constant glycosuria with normal glomerular filtration rates and in conditions of normoglycemia.[29]

The observation that the maximum tubular glucose reabsorption rate and the saturation capacity are markedly affected by chronic hyperglycaemia[22,30,31] has provided the basis for a novel approach in the treatment of diabetes. Exposure to hyperglycemia is reportedly accompanied by an increase in tubular *TmG*, reflecting enhanced maximum glucose transport capacity and reabsorption. As a consequence, the appearance of glucose in the urine tends to occur at plasma glucose concentrations above the normal renal threshold of 180-200 mg/dl in patients with diabetes.

This adaptation was first reported by Farber *et al.* in 1951 who showed that the "splay" for maximal glucose reabsorption (*Tmax*) was expanded and the renal threshold augmented in patients with type 2 diabetes by 20-40%.[30] Two decades later, Morgensen[22] described similar findings in patients with type 1 diabetes. More recent data derived from *in vitro* studies using cultures of proximal renal tubular cells collected from urine samples of subjects with and without diabetes fully supported these observations. In these experiments proximal tubular cells from patients with diabetes were shown to have increased mRNA expression and SGLT-2 protein content. Moreover, using a radio-labelled glucose analogue functional assay these cells also exhibited an elevated glucose transport capacity.[31] Altogether, these results were interpreted as an indication that there is a *maladaptive* response of the kidney to hyperglycemia in diabetes mellitus. It has been speculated that by increasing glucose reabsorption rates, the kidney helps to maintain the abnormal status of hyperglycemia, which may in turn lead to further *maladaptation*.

Inhibitors of tubular glucose reabsorption

The possibility that the diabetic kidney perpetuates hyperglycemia gave rise to the notion that agents capable of inhibiting renal glucose reabsorption might be useful in lowering blood glucose.[32-34] As originally envisioned, SGLT inhibitors would reduce the tubular capacity for glucose reabsorption and promote glucosuria at lower plasma glucose levels (*low Tm glucose*). This hypothesis was tested with phlorizin, a compound extracted from the root bark of apple tree with presumed anti-diabetic properties due to its known *in vitro* inhibition of tubular glucose transport.

In 1987, Rosettti *et al.*[32] published data indicating that hyperglycemia, which developed in the fasting and fed state in rats after partial pancreatectomy, could be reversed with once daily intra-peritoneal injection of phlorizin. Following the discontinuation of the injections hyperglycemia was again detected, thus confirming that phlorizin was responsible for the improvement. These findings suggested that with adequate chemical blockade of the SGLT transport activity, the renal threshold for glucose reabsorption could be decreased and better glycemic control achieved in patients with diabetes.

Although there was some excitement surrounding these findings, the lack of selectivity of phlorizin, the associated adverse gastro-intestinal effects and the uncertainty regarding the consequences of the induced glucosuria dampened the initial enthusiasm for the clinical development of this agent. Furthermore, the realization that phlorizin was quickly degraded by *lactase-phlorizin hydrolase*, was poorly absorbed in the intestines and had very low bioavailability further halted any investigation using this nonspecific SGLT inhibitor in humans.[34]

A search for SGLT-2 inhibitors that were more selective and more resistant to intestinal degradation and had higher plasma bioavailability was launched. Many agents with SGLT inhibitor properties were obtained by techniques capable of chemically modifying the parent compound phlorizin ("second generation agents"). Initially *in vitro* experiments in cultured cell lines expressing human SGLT-1 and SGLT-2 transporters were conducted to determine the degree of selectivity of any given SGLT inhibitor using radio-ligand binding assays.[35-37]

Following a series of investigations a high degree of selectivity inhibition of SGLT-2 was initially shown for "empagliflozin" (*L-chloro-4-(β-D-glycopyranos-1-Yl)-2-[4-((S)-tetrahydrofuran-3-Yl-oxy)-benzyl-benzene*). Numerous other compounds were tested in a similar fashion and *in vivo*

animal studies confirmed that these "second generation" agents were minimally degraded in the intestines and were only subject to a mild-to-modest total plasma clearance. Adequate bioavailability in the range of 33 to 80% was documented for most of these "second-generation" compounds developed.[35-37]

The specificity of some of these agents *in vivo* was questioned by the recent findings in humans revealing that orally ingested selective SGLT-2 inhibitors must first interact with the SGLT-1 transporters at the brush-border membrane of enterocytes.[38] Because the enterocytes containing the SGLT-1 transporters are exposed to a greater load of the SGLT-2 specific inhibitors inside the gut, the binding affinity and thus, the selectivity is lost. As a result, the activity of the sodium-glucose/galactose co-transport process as a whole is reduced and there is a transient decline in the intestinal absorption of these sugars. The selectivity for the renal SGLT-2 transporters is nevertheless regained after partial splanchnic clearance and with lower circulating plasma drug levels.

Once selective inhibitors reach the kidney via the systemic arterial blood circulation, they bind avidly to SGLT-2 transporters in the luminal tubular membrane. In contrast, at much lower concentrations the binding affinity of these agents for the SGLT-1 transporter located downstream in the same area is severely diminished and no inhibition of the SGLT-1 activity in the kidney is detected.[35] Following an insulin-independent decline in blood glucose, SGLT-2 inhibition is also accompanied by mild improvement in insulin sensitivity,[38,39] which represents an additional mechanism by which these agents contribute to glycemic control in patients with type 2 diabetes. Moreover, recent findings reported in a SGLT-2 knockout mouse model provide evidence for an alternative approach to improving glycemic control and reducing insulin sensitivity with preservation of beta-cell function, by simply reducing the renal threshold and promoting renal glycosuria.[40]

Clinical pharmacology and pharmacokinetics

The maximum inhibitory effect achievable on the renal glucose re-uptake with the use of selective blockade of the tubular SGTL-2 transporter activity in humans has been reported at 30-50%.[41,42] It has been postulated that the efficacy of these agents is somewhat limited, in part because of the competitive nature of the inhibitory binding process. There is also the possibility that very low levels of the active drug reach the tubular luminal membrane, the main site of the drug action. Finally, and perhaps most importantly, the extent to which a compensatory enhancement in the glucose reabsorption capacity of the SGLT-1 co-transporter or by a yet unidentified tubular glucose transport system, located downstream contributes to the low effectiveness of these agents has not been defined.[15,19] Once plasma steady-state concentrations of the SGLT inhibitor are reached (4 to 5 days), the total amount of glucose excreted in the urine is around 50-80 grams per day. This results from the partial blockade of the SGLT-2 co-transporter and reflects a shift to the left in the maximum tubular transport capacity with a substantial decrease in the renal threshold [*TmG*].[38,41]

Clinical observational studies conducted in healthy non-diabetic and in diabetic subjects have indicated that the appearance of glucosuria can be detected within 8-16 weeks after the oral intake of the first dose of an SGLT-2 inhibitor.[43,44] Actually, it has been estimated that following a short therapy period with SGLT-2 inhibitors glucosuria can be detected both in subjects with and without diabetes at plasma glucose values ranging anywhere from 40-120 mg/dl.[38] This remarkable shift in the renal threshold for glucose reabsorption contributes to a significant decrease in circulating plasma glucose, which is accomplished with a daily loss of 200-320 calories.

These changes combined can provide much desired benefits for obese patients with diabetes. The decline in plasma glucose concentration following the use of SGLT inhibitors has been recently reported to be attenuated by a rise in plasma glucagon accompanied by an elevation in endogenous glucose production.[39,45] These findings were documented in subjects who had received SGLT-2 inhibitors for a short period of time and who had experienced a drop in plasma glucose levels, but with no evidence of hypoglycemia. These intriguing observations have raised new questions regarding potential interactions of the kidney and liver in glucose regulation and counter-regulation, and will require confirmation.

Following oral administration SGLT-2 inhibitors are rapidly absorbed with peak plasma

concentrations (median T_{max}) occurring 1-2 hours post-dose. Plasma C_{max} and the area under the curve (AUC) increase in a dose-proportional manner with apparent terminal half-life ($t_{1/2}$) of varying from 10 up to 13 hours. The active drug reaches a steady-state usually within 4 to 5 days.[37,38,41] The major metabolic elimination of SGLT-2 inhibitors is via hepatic glucuronidation and the inactive metabolites are released into peripheral circulation. There is minimal CYP3A4-mediated oxidative metabolic degradation. Thus, clinically relevant effects of other drugs on the pharmacokinetics of SGLT-2 inhibitors via cytochrome P450 are unlikely to occur. Also, since the CYP450 enzyme system is not induced and is only minimally attenuated by SGLT-2 inhibitors only negligible changes in drugs utilizing the same hepatic metabolic processes have been reported.

In contrast, a decrease in total exposure to active SGLT-2 inhibitors occurs when UGT (glucuronosyl transferase) inducers, such as rifampin, phenytoin and phenobarbital are co-administered. Plasma digoxin levels tend to increase and require closer monitoring when used in combination with SGLT-2 inhibitors. When using oral radio-labeled SGLT-2 inhibitors in normal healthy volunteers nearly 50% of the active drug is recovered in feces together with minor amounts (less than 10%) of some inactive metabolites; less than 1% of the oral dose is excreted intact in the urine.[46]

Safety and efficacy of SGLT inhibitors

The fact that selective inhibitors of SGLT-2 lower plasma glucose concentration via an insulin-independent manner, and thus with minimal risk of hypoglycemia, combined with the potential to induce simultaneous body weight loss has generated considerable clinical interest.[47-51] Several SGLT-2 inhibitors are currently in development and some have already been approved for use in the treatment of patients with type 2 diabetes. Results from a few selected clinical trials are summarized on Table 1.

Most studies have shown consistent and unequivocal improvements in glycemic control in a variety of diabetic patients with a decline in both fasting and postprandial plasma glucose. The mean reported decrease in the glycosylated hemoglobin (HbA_{1c}) values was approximately 1.0%, which was initially documented over a period of 6 months. More recent preliminary data have confirmed that these changes can be sustained with safety up to 4 years.[52] The degree of glycemic control attained with SGLT-2 inhibitors was shown to be either comparable or superior to anti-diabetic agents routinely recommended in standard practice.[49-51]

The exact placement of SGLT-2 inhibitors in algorithms designed to guide management of diabetes remains undetermined and is likely to be somewhat controversial.[53-55] These drugs are known to be effective as monotherapy and, even though the decrease in blood glucose can be attained within a wide range of plasma glucose concentration, whether early in the disease (or in pre-diabetes) the tubular reabsorption capacity is abnormally enhanced has not been fully determined. Moreover, with limited clinical experience and considering the added cost this class of drugs is more likely to be used later, rather than earlier in the treatment of type 2 diabetes. The possibility nevertheless of combining these agents with other well-established anti-diabetic drugs, oral and injectables seems more reasonable. Taking into account the unique mechanism of action of SGLT-2 inhibitors, maybe they are best if indicated in patients with poorly-controlled type 2 diabetes in whom oral treatment has been exhausted and the initiation of injectable agents or insulin replacement therapy is eminent.

These suggested therapeutic options are not based on firm scientific evidence and represent but one viewpoint. The ultimate decision as to when and how to best use SGLT-inhibitors during the course of diabetes treatment will require additional data as well as the experience acquired over a prolonged period of time.

Body weight loss was anticipated and has occurred in almost all diabetic patients who received therapy with SGLT-2 inhibitors in pivotal clinical trials.[47-52] The usual amount of body weight lost was reported in the range of 2 to 4 kilograms over a period of observation of 6 months, with only a few outliers. Interestingly, studies using other drugs that promote body weight usually provide results with noticeable individual variability, whereas SGLT-2 inhibitors tend to induce nearly equal body weight loss in just about everyone treated. A recent study indicated that the majority of the weight reduction was due to the loss of body fat mass, ~50% each in the abdominal and subcutaneous fat depots, with minimal changes in lean body mass.[56]

Table 1 Reported clinical efficacy of various SGLT-2 inhibitors used in pivotal clinical trials in the treatment of type 2 diabetes

SGLT-2 inhibitor	Placebo/comparator	Duration of study	Baseline HbA_{1C}	Δ HbA_{1C}^	Δ Body^ Weight (lbs)
Canagliflozin 100 mg 300 mg	*vs.* PLACEBO	26 weeks	~8.0% ~8.1%	-0.91%* -1.16%*	-4.2* -6.4*
Canagliflozin 300 mg	*vs.* SITAGLIPTIN (+ MET & SU)	52 weeks	~8.1%	-0.37%**	-5.2*
Canagliflozin 100 mg 300 mg	*vs.* PLACEBO (+ Insulin & AHA)	26 weeks	~8.3%	-0.69%* -0.73%*	-1.9* -2.4*
Dapaglifozin 10 mg	*vs.* MET alone *vs.* COMBO	24 weeks	~8.1%	-1.44%@ -1.98%@	-3.2@ -4.8@
Dapaglifozin 10 mg	*vs.* SU (+ MET)	52 weeks	~7.7%	-0.18%**	-2.5**
Dapaglifozin 10 mg	*vs.* SU (+ MET)	208 weeks (4 years)	~7.7%	-0.30%*	-5.6*
Empaglifozin# 5 mg 10 mg 25 mg	*vs.* PLACEBO	16 weeks	~7.9%	-0.24%* -0.52%* -0.50%*	-3.0* -5.2* -4.8*
Ipraglifozin# 50 mg	*vs.* PLACEBO	16 weeks	~8.3%	-1.10%*	-5.2*

Δ HbA_{1C} and Δ Body Weight: Represent mean values, degree variation not provided; ^ statistically significant with $p < 0.05$, unless otherwise indicated (# p values not available); *placebo-subtracted values; ** comparator-subtracted values; @ changes from baseline.

The stabilization of the body weight achieved 6 months after the initiation of therapy, which has been confirmed to persist up to 4 years with the continued use of SGLT-2 inhibitors is regarded as a remarkable accomplishment and provides further reassurance to those who manage obese type 2 diabetic patients.[52] Whether a later compensatory increase in appetite and/or a change in energy expenditure will occur in response to the loss of calories in the urine over longer periods of time should not be entirely discarded. As a reminder, these agents are not approved for the sole treatment of overweight and obese individuals who do not have a diagnosis of type 2 diabetes.

The low incidence of hypoglycemia is a clinically relevant and important characteristic associated with the use of SGLT-2 inhibitors in the management of type 2 diabetes. This results from the fact that the mechanisms underlying the glucose-lowering effect of a partial blockade of the tubular glucose re-uptake are insulin-independent and do not involve direct changes in insulin secretion.

Furthermore, the rate of urinary glucose excretion is proportionate to the circulating plasma glucose concentration (i.e., glucose-dependent glucose excretion) and thus, it becomes almost negligible in conditions of very low plasma glucose concentrations. In contrast, when SGLT-2 inhibitors are used in combination with insulin secretagogues (sulfonylurea, meglitinides) or together with insulin injections the risk for hypoglycemia is magnified.[47-52] There is no current approved indication for the co-administration of SGLT-2 inhibitors with insulin therapy in type 1 diabetes, just as there are no data on the safety and efficacy of these agents in pediatric patients under 18 years of age.

Some important and a few unexpected findings have been reported in patients with type 2 diabetes who were exposed to various SGLT-2 inhibitors in clinical trials (Table 2). Following initial treatment a transient period (days to weeks) of polyuria, urinary frequency with increased thirst, often characterized as a simple state of dehydration was described in 3-5% of all study subjects.[50,51] Two-thirds of these individuals had symptoms of postural dizziness and most of them had documented orthostatic hypotension. The majority recovered uneventfully, presumably because blood volume and fluid balance were appropriately corrected by alternate renal and some other mechanisms. Of note, dehydration and orthostatism was more common in elderly diabetic patients who were taking anti-hypertensive drugs and/or diuretics.

Table 2 Commonly reported adverse events of various SGLT-2 inhibitors used in pivotal clinical trials in the treatment of type 2 diabetes

SGLT-2 inhibitor	Increased Urinary Frequency	Increased Thirst	Hypotension	Hypoglycemia	Urinary Tract Infections	Genital Infections
Canagliflozin 100 mg (n = 3,092) 300 mg (n = 3,085)	174 (5.6%) 177 (5.7%)	80 (2.6%) 70 (2.5%)	20 (< 1.0%) 30 (< 1.0%)	71 (2.3%) 104 (3.4%)	171 (5.5%) 175 (5.7%)	510 (16.3%) 545 (18.1%)
Dapaglifozin 5 mg (n = 1,145) 10 mg (n = 1,193)	0 1 (< 1.0%)	0 1 (< 1.0%)	5 (0.4%) 5 (0.4%)	25 (2.2%) 35 (3.0%)	149 (13.0%) 131 (11.0%)	155 (13.5%) 181 (15.1%)
Empaglifozin 10 mg (n = 495)	12 (2.5%)	1 (< 1.0%)	N/A	N/A	19 (4.0%)	49 (10.0%)
Ipraglifozin 50 mg (n = 62)	N/A	N/A	N/A	N/A	1 (4.0%)	2 (< 1.0%)

N/A: Not available.

Despite the transient nature of these acute hemodynamic events, greater caution and a special attention to this vulnerable population will be required by prescribing physicians and health care providers. For reasons that are not entirely clear, a slight and consistent decrease in systolic and diastolic blood pressure has been recorded in nearly all diabetic patients treated with SGLT-2 inhibitors for at least 6 months.[47-52]

Whether this potential beneficial effect can be related to changes in blood volume and hydration status and/or to a direct or indirect vascular dilation property of SGLT-2 inhibitors remains undetermined.

Rare cases of mild hyperkalemia following the administration of SGLT-2 inhibitors have been reported, primarily in patients with some degree of renal insufficiency.[57] Nearly all diabetic patients who experienced serum potassium elevations were using potassium-sparing diuretics, angiotensin-converting enzyme inhibitors or angiotensin receptor blocking agents. We speculate that by further altering the tubular-glomerular feedback loop with the reduction of the sodium-glucose reabsorption in proximal tubules these agents may exacerbate an underlying hyporeninemic-hypoaldosteronism state, commonly seen in type 2 diabetes.[58] No serious clinical consequences however have yet been registered in association with hyperkalemia induced by SGLT-2 inhibitors.

Considering the recommendations for the use of SGLT-2 inhibitors are limited to lower doses and should be given only to diabetic patients with estimated glomerular filtration rate (eGFR) above 30 ml/min/1.73 m^2, the occurrence of hyperkalemia is expected to be a rare event. Because of pure inefficacy, these agents are not indicated in patients with advanced end-stage renal disease and those on renal dialysis. In case of inadvertent drug overdose and intoxication, SGLT-2 inhibitors cannot be removed from the circulation efficiently by hemodialysis. Almost nothing is known about untoward effects associated with acute elevations and tissue accumulation of the native SGLT-2 inhibitor compounds and their metabolites.

In patients with mild-to-moderate hepatic insufficiency, nonetheless there is no need for adjustments in dose, although the safety and efficacy of these SGLT-2 inhibitors have never been tested in patients with severe hepatic insufficiency. Also, the use of SGLT-2 inhibitors is contra-indicated during pregnancy and in lactating diabetic women, since newborn animals exposed to this agent exhibit a multitude of kidney and urogenital malformations.[47]

According to results from one large pivotal study presented earlier to the Food and Drug Administration (FDA) Advisory Committee, 9 cases of bladder cancer out of 5,501 patients (0.16%) were reported in association with the use of dapagliflozin, as opposed to only one patient out of 3,184 (0.03%) treated with placebo/comparator.[47] During careful analyses of the data it was noted that all bladder cancers occurred in male patients and that 7 of all 10 patients with the diagnosis had had hematuria prior to the initiation of the study treatment. Furthermore, eight patients with bladder cancer were current or former smokers, five of them were diagnosed at < 6 months from the start of dapagliflozin therapy, and none diagnosed with treatment longer than 24 months. In the same clinical

trials, breast cancer developed in ten out of 2,531 women (0.40%) treated with dapagliflozin *vs*. three out of 1,359 women (0.22%) in the placebo/comparator groups. All breast cancers were recorded in female patients above the age of 50 years and, in fact 10 of 13 cases occurred in women above the age of 60 years.

The diagnosis was confirmed in women exposed to dapagliflozin for less than one year and in two cases the presence of breast cancer was documented within the first eight weeks of treatment. Based on these observations, the FDA concluded that there were too few events to establish causality, a decision supported by the fact that no carcinogenicity or mutagenic signal has been described during pre-clinical animal studies. Although the accumulation of large amounts of glucose in the bladder urine over time cannot be entirely rule out as a putative carcinogenic factor, it is reassuring to know that SGLT-2 transporter proteins are not expressed either in human bladder or in breast tissue.[59]

There was a noticeable increase in the incidence of urinary tract and genital infections in patients with type 2 diabetes who were treated with SGLT-2 inhibitors reported in all clinical trials.[48-52] Nearly all infections were limited to the lower urinary tract and were reported in ~8-13% of participants receiving SGLT-2 inhibitors, but only in ~3-8% of those randomized to either placebo or a comparator drug. Similarly, genital infections developed in ~12-15% of patients taking SGLT-2 inhibitors, whereas those using placebo or a comparator drug had an incidence no higher than ~5%.

These observations were derived from studies that included more than 10,000 patients with type 2 diabetes followed by at least 2 years of exposure to various SGLT-2 inhibitors.[47] Women, especially those with a positive past medical history were more commonly affected, although the vast majority of infections resolved with standard treatment, did not require hospital admissions, and recurrences were infrequent. Actually, many of the participants who developed urinary and genital infections elected to continue with the treatment, particularly those in whom glucose control and body weight loss were apparent. Less than 2% of all patients treated with SGLT-2 inhibitors who developed genital mycosis, namely balano-postitis, were uncircumcised men.[47]

Inasmuch as the potential for long-term adverse effects of SGLT-2 inhibitors on kidney function is unpredictable, we can take some comfort on the observations that individuals with "familial renal glucosuria" are essentially disease-free and live near-normal lives.[60]

This is an autossomal recessive disorder with either complete deficiency or decreased affinity for the SGLT-2 co-transporter protein. The genetics of "familial renal glucosuria" have been studied extensively and 21 different mutations in the gene for SGLT2 were detected. Homozygous individuals tend to have glycosuria that varies from 15 up to 200 g/day, whereas pure heterozygous family members have either mild glycosuria or none at all. Because this condition is characterized by persistent urinary glucose excretion, even within the normal range of plasma glucose concentration these individuals have difficulty maintaining body weight.

There is no evidence of renal glomerular or tubular dysfunction, as assessed by kidney function and renal histological evaluations. Hypoglycemia is uncommon and the incidence of diabetes mellitus, chronic renal insufficiency and urinary tract infections is comparable to the general population. The diagnosis of "familial renal glucosuria" must be distinguished however from other complex tubular disorders that can be associated with some morbidity.[60]

There is no clinical evidence that the use of SGLT-2 inhibitors in patients with type 2 diabetes is associated with improved cardiovascular outcomes. A long-term trial is underway to address this question and we shall soon have an answer. In the meantime, the available data indicate that these drugs are cardiovascular safe and so far have not been accompanied by increases in cardiovascular events.[47]

The observation that there is a slight but consistent decrease in systolic and diastolic blood pressure sustained for up to 104 weeks contrasts with the slight elevation in plasma low-density lipoprotein (LDL) cholesterol particles described in most clinical studies, which makes it even more difficult to predict the final results of the ongoing cardiovascular outcomes trial.

In summary, the kidney plays an important role in glucose homeostasis, contributes to glucose regulation and counter-regulation, and in sparing glucose also helps to preserve the energy balance. These remarkable functions are achieved by an active proximal tubular mega-transport system that promotes complete glucose reabsorption and by the minimal intrinsic

glucose production that often matches renal glucose utilization. Chronic hyperglycemia is associated with an increase in renal threshold and renal glucosuria occurs at higher than normal plasma glucose concentrations.

As a consequence, specific inhibition of the high capacity renal sodium-glucose co-transporter (SGLT-2) has emerged as a potential pharmacological intervention, which by decreasing tubular glucose reabsorption rates induces glucosuria and reduces blood glucose levels. In addition, continuous loss of calories in the urine is accompanied by a sustained decrease in body weight/fat in obese patients with type 2 diabetes. Initial observations in pre-clinical studies and in clinical trials have raised expectations for the utilization of SGLT-2 inhibitors in the treatment of type 2 diabetes. The data collected so far demonstrating a clinically significant glucose-lowering effect, body weight loss and negligible risk of hypoglycemia in patients treated with this novel class of drugs are very consistent.

The development of transient polyuria with dehydration and the occasional hypotension, particularly in elderly diabetic patients is of concern. The high frequency of urinary tract infections and genital mycosis requires close monitoring. Lower doses of SGLT-2 can be used safely in individuals with mild-to-moderate, but not in those with severe and end-stage renal insufficiency. Whether there is any long-term damage to the kidney is unknown and cardiovascular benefits are yet to be demonstrated with the use of these novel agents. Also, adverse effects on bone and mineral metabolism have not been sufficiently investigated. Some SGLT-2 inhibitors are now approved for the treatment of type 2 diabetes, either as monotherapy or in combination with other anti-diabetic medications. As long as patients can tolerate these agents, given all that we know today, SGLT-2 inhibitors if used with caution and in the proper patient may provide an additional safe and efficacious therapeutic option in the management of type 2 diabetes.

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