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Pharmacological aspects of the safety of gliflozins

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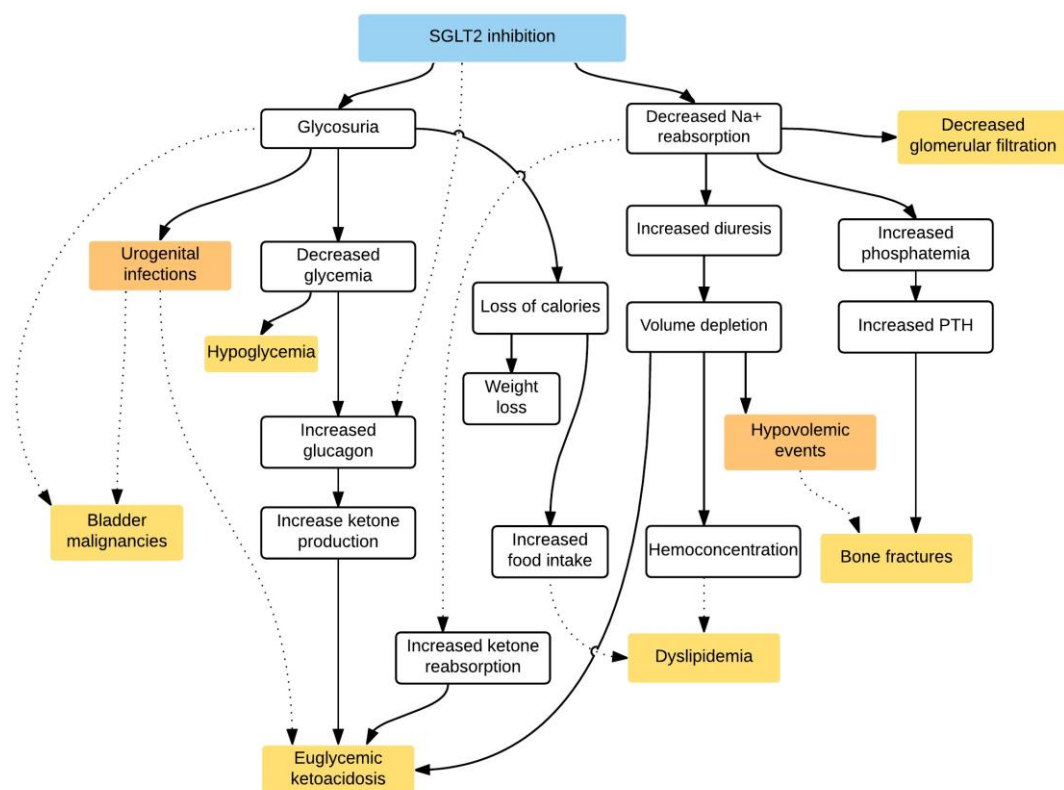
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Graphical abstract



Main effects and suspected adverse reactions secondary to inhibition of sodium-glucose transporter 2 (SGLT2) by gliflozins.

Abstract

Sodium-glucose transporter 2 (SGLT2) inhibitors, also known as gliflozins, are a new class of orally active drugs used in the management of type 2 diabetes. By inhibiting the SGLT responsible for the reabsorption of glucose from the kidney, their use aims primarily to induce glycosuria and, as a consequence, lower glycemic levels. However, their specific mechanism of action involves other pharmacodynamic consequences including potentially harmful adverse reactions. This manuscript reviews the physiological and pharmacological background behind inhibition of SGLTs, and discusses the pharmacological aspects of the safety of gliflozins.

Chemical compounds studied in this article

Dapagliflozin (PubChem CID: 9887712); canagliflozin (PubChem CID: 24812758); empagliflozin (PubChem CID: 11949646); ipragliflozin (PubChem CID: 10453870); tofogliflozin (PubChem CID: 46908929); phlorizin (PubChem CID: 6072).

Introduction

Sodium-glucose transporter 2 (SGLT2) inhibitors are a new class of orally active drugs used in the management of type 2 diabetes. The first SGLT2 inhibitor to be authorized was dapagliflozin in Europe in 2012, and nowadays this class, also known as the “gliflozins”, includes other drugs such as canagliflozin, empagliflozin, ipragliflozin, luseogliflozin, tofogliflozin, etc.. By inhibiting the SGLT responsible for the reabsorption of glucose from the kidney, their use in patients with type 2 diabetes aims primarily to increase glycosuria and, as a consequence, lower glycemic levels. However, their specific mechanism of action involves other pharmacodynamic consequences; some are beneficial (such as the increased insulin sensitivity or the decrease in systolic blood pressure) but others are potentially harmful adverse reactions. In this manuscript, we review the physiological and pharmacological background behind inhibition of SGLTs, and discuss the pharmacological aspects of the safety of gliflozins.

Sodium-glucose transporters

Glucose transport is a crucial mechanism in glycemic regulation and is provided mainly by two types of protein: the GLUTs (facilitated transport) and the SGLTs (active transport). These proteins belong to the SLC (Solute Carriers) gene family and are coded by the gene subfamilies SLC2A and SLC5A, respectively. The SLC5A subfamily carriers is represented by 12 members which all code for transporters of various substrates (sugars, ions, vitamins, fatty acids, choline...), except for SLC5A4 (SGLT3) which is a glucose-gated ion channel considered as a glucose sensor in neurons and muscle.¹ The members of the SLC5A family coding for transporters that involve sugars and their distribution in human tissues are presented in Table 1.

Table 1. Human SLC5A gene family carriers that involve sugars.^{1,2}

Gene (locus)	Protein	Transport type	Human tissue distribution
SLC5A1 (22q12.3)	SGLT1	Cotransporter of glucose or galactose with Na ⁺ , (H ⁺) Uniporter of urea and water	Small intestine, trachea, kidney, heart, brain, testis, prostate
SLC5A2 (16q11.2)	SGLT2	Cotransporter of glucose with Na ⁺	Kidney, brain, liver, thyroid, heart muscle, salivary glands
SLC5A3 (21q22.12)	SMIT1	Cotransporter of myoinositol (glucose) with Na ⁺	Brain, heart, kidney, lung
SLC5A4 (22q12.3)	SGLT3	Glucose activated Na ⁺ (H ⁺) channel	Small intestine (cholinergic enteric neurons), skeletal muscle, kidney, uterus, testis, lung, brain, thyroid
SLC5A9 (1p33)	SGLT4	Cotransport of mannose, fructose or glucose with Na ⁺	Kidney, small intestine, brain, liver, heart, lung, trachea, uterus, pancreas
SLC5A10 (17p11.2)	SGLT5	Cotransport of mannose, fructose or glucose with Na ⁺	Kidney cortex
SLC5A11 (16p12.1)	SGLT6	Cotransport of myoinositol, chiro-inositol with Na ⁺	Thyroid, brain, heart, muscle, spleen, liver, lung

SGLTs are transmembrane glycoproteins with 14 transmembrane helices. Their average molecular weight is approximately 75 kD (60 to 80kD, 580 to 718 residues). Relative to SGLT1, amino acid homology of other SGLTs range from 50% to 70% (59% for SGLT2).¹

The two most studied members of this family are SGLT1 and SGLT2. SGLT1 is mainly expressed in the small intestine enterocytes where it transports glucose and galactose from the gut lumen across the brush border membrane. Mutations of the SGLT1 gene (SLC5A1) cause glucose-galactose malabsorption, an autosomal recessive disease in which the presence of lactose, glucose and galactose in the diet is responsible for a severe osmotic diarrhea and dehydration. SGLT1 is also expressed in the renal proximal tubule (S3 segment) and glucose-galactose malabsorption is also associated with a minor glycosuria.^{2,3} SGLT2 is predominantly expressed in the brush border membrane of the epithelial cells of the renal proximal tubule (S1 and S2 segments) where it is responsible for the main part of renal glucose reabsorption.⁴ Mutations in the gene encoding SGLT2 (SLC5A2) are associated with familial renal glycosuria, a generally benign condition characterized by renal loss of glucose without evidence of hyperglycemia or other renal dysfunction.² In patients with familial renal glycosuria, homozygous mutations are generally associated with a glycosuria exceeding 10-100 g/1.73 m²/day, whereas heterozygous mutations generally result in glycosuria lower than 10 g/1.73 m²/day.⁵ In the renal proximal tubule, SGLT2 is responsible for 90% of glucose reabsorption whereas SGLT1 transports the remaining 10% (Figure 1).⁴ SGLT1 and SGLT2 are thus responsible for the renal threshold for glucose, the critical glycemic level that determines the presence or absence of glucose in the urine. In healthy adult, glucose is only found in the urine when the maximum capacity of the tubule to reabsorb glucose is attained *i.e.* when plasma glucose concentrations exceeded a threshold of approximately 200 mg/dL (11.0 mmol/L).⁶

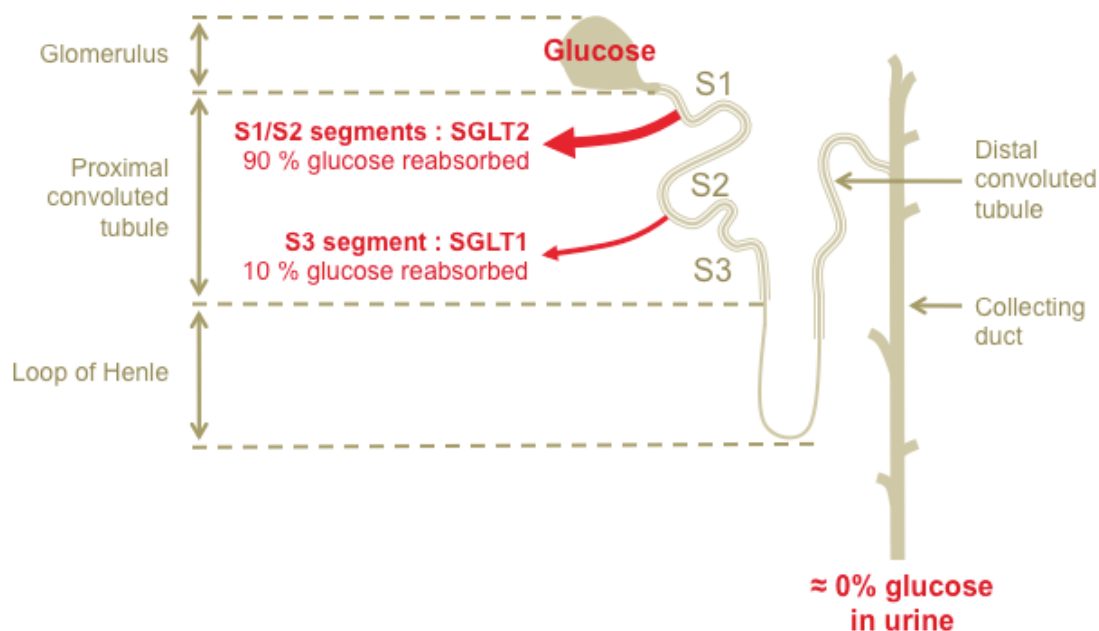


Figure 1. Reabsorption of glucose by the kidney in healthy adult.

SGLT1 and SGLT2 operate in a monomeric fashion, the rate and the direction of glucose transport simply depends on the sodium electrochemical potential gradient across the membrane. First, sodium and glucose bind to the transporter on one side of the membrane, then,

the protein conformation changes, and finally the substrates are released at the other side.¹ There is a fixed stoichiometry between sodium and sugars, for one sodium ion transported, SGLT1 carries two molecules of glucose or galactose whereas SGLT2 only carries one molecule of glucose.⁷ SGLT2 shows a high capacity and a low affinity for glucose ($K_m = 2 \text{ mM}$) whereas SGLT1 shows a low capacity and a high affinity ($K_m = 0.4 \text{ mM}$).⁶ As presented in Figure 2, the cotransport of glucose and sodium into the proximal tubule cell is dependent of a sodium gradient produced by Na^+/K^+ ATPase pumps in the basolateral membrane of the epithelial cell.⁸ Once glucose has penetrated the proximal tubule cell, it is passively transported to the interstitium by GLUTs (primarily GLUT2) according to its concentration gradient.²

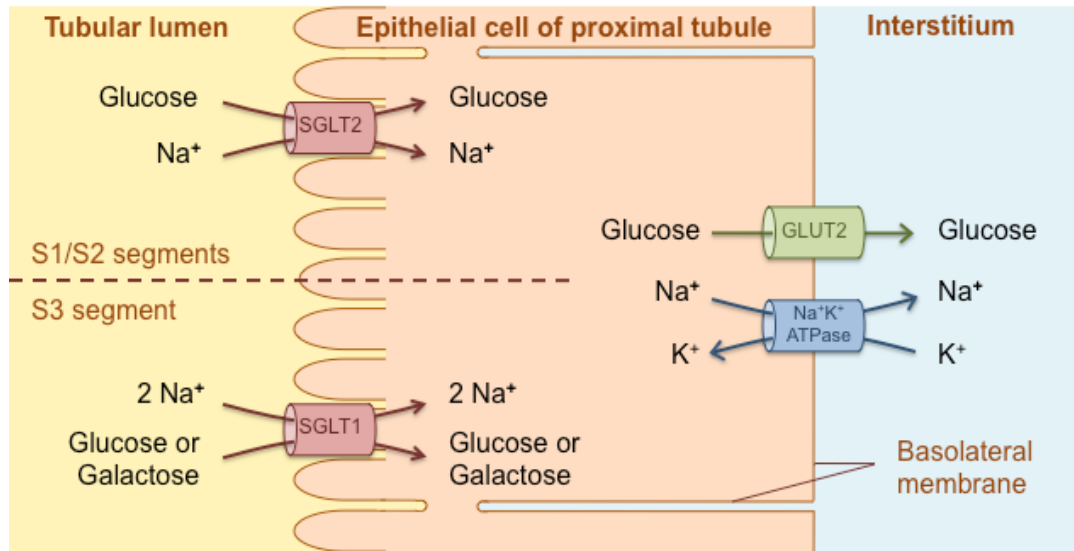


Figure 2. Glucose reabsorption from the proximal tubule. The cotransport of glucose and sodium into the proximal tubule cell is dependent of a sodium gradient produced by Na^+/K^+ ATPase pumps in the basolateral membrane.

In patients with diabetes, an upregulation of SGLT2 is observed and results in an increased capacity for the reabsorption of glucose. This phenomenon aggravates the hyperglycemia by reabsorbing the excessive amounts of glucose that would be excreted into the urine by the kidneys of healthy people.⁹ Among other SGLTs, it is noteworthy that SGLT3 has been identified in the renal tubule.¹⁰ SGLT3 does not transport glucose into cells but acts as a sodium channel stimulated by glucose, which can cause an increase in sodium reabsorption, an effect already increased in type 2 diabetes.¹¹

The development of SGLT inhibitors

The history of SGLT inhibitors started in 1835, when the glycoside phlorizin (a type of flavonoid) was isolated from the apple tree bark. According to its bitter taste that was similar to other tree extracts used as antipyretics, it was firstly administered to patients with infectious diseases such as malaria.¹² In 1886, von Mering discovered that phlorizin ingestion causes glycosuria and, a century later, studies of phlorizin's mechanism of action led to the characterization of sodium-glucose transporters.^{13,14} In 1987, phlorizin was studied in diabetic rats and demonstrated its efficacy to reduce glycemic level and restore insulin sensitivity.¹⁵ However, phlorizin, which is an inhibitor of both SGLT1 and SGLT2, was not developed for human use due to limited oral bioavailability and gastrointestinal side effects (explained by

inhibition of glucose absorption in the small intestine).¹⁶ Indeed, phlorizin is an O-glycoside which is mainly hydrolyzed by intestinal glycosidases. Since then, because the majority of the glucose reabsorption by the kidney is mediated via the SGLT2, the gliflozins have been developed. These drugs are presented as more specific SGLT2 inhibitors and are C-glycosides that are resistant to gastrointestinal beta-glycosidases (Figure 3).¹⁷

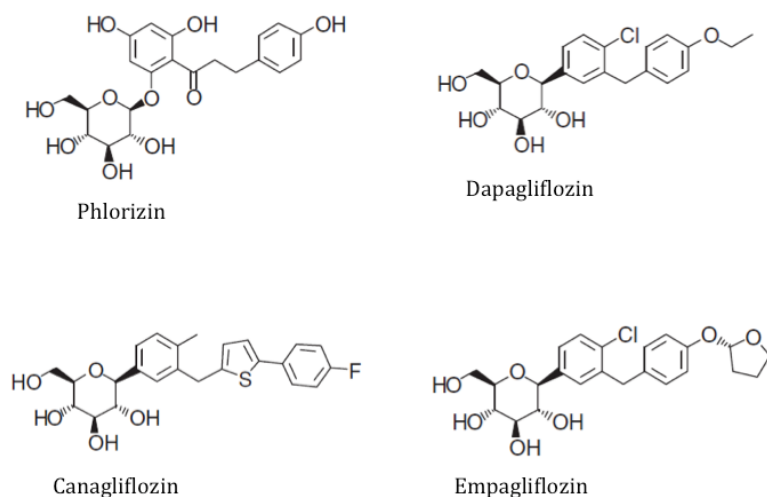


Figure 3. Chemical structures of phlorizin, canagliflozin, dapagliflozin and empagliflozin.

Pharmacokinetic and pharmacodynamic characteristics

Pharmacokinetic parameters of the main authorized gliflozins are: a relatively good oral bioavailability (dapagliflozin 78%, canagliflozin 65% and empagliflozin >60%), a T_{max} reached within 1 to 2 hours, a relatively high plasma protein binding (dapagliflozin 91%, canagliflozin 98% and empagliflozin 86%), a volume of distribution ranging from 74 L for empagliflozin to 118-119 L for dapagliflozin and canagliflozin, a half-life of approximately 12 hours, no significant food or drug pharmacokinetic interaction, an extensive hepatic metabolism into inactive glucuronidated conjugates, and an elimination primarily in urine and feces as inactive metabolites with less than 2% eliminated unchanged in urine, except for empagliflozin for which approximately 18% is excreted unchanged in urine.^{18,19}

The mechanism of action of SGLT inhibitors implies binding to the transmembrane SGLT by mimicking a sugar conformation resulting in the blockade of the glucose and sodium transport cycle.²⁰ However, the way SGLT inhibitors reach their site of action is not clearly determined. Gliflozins only inhibit the reabsorption of the filtered glucose by 30% to 50% (whereas SGLT2 is responsible for 90% of renal glucose reabsorption). In addition, the urinary glucose excretion is maintained during a long time after the plasma levels of the SGLT2 inhibitors have decreased. These two observations indicate that glomerular filtration may not be the only way for SGLT2 inhibitors to reach renal SGLTs and this questions the potential role of other mechanisms: i) renal secretion and/or reabsorption of gliflozins, ii) a site of secretion that could be downstream to where SGLT2 are mainly expressed (S1/S2 segments), iii) potential superpotent minor metabolites, iv) augmented capacity of SGLT1 (in S3 segment) secondary to complete SGLT2 inhibition, or other compensatory mechanisms.^{21,22} Furthermore, action of SGLT inhibitors from inside the cell cannot be excluded.

Inhibiting the renal SGLT2 lowers the physiological renal threshold for glucose by reducing the reabsorption of filtered glucose which in turn increases the excretion of glucose by the kidneys.⁶ In patients with type 2 diabetes treated by gliflozins, increased urinary glucose excretion (70-100 g/day) results in an insulin-independent lowering of elevated plasma glucose concentrations. A meta-analysis of 58 gliflozin trials has shown that mean HbA1c reduction versus placebo was -0.66% (95% confidence interval [CI]: -0.73 to -0.58) but was not significantly more favorable versus active comparator (-0.06%, 95% CI: -0.18 to 0.05).²³ Gliflozin-induced glycosuria is also responsible for an osmotic diuretic effect which leads to a mean reduction in systolic blood pressure of -4.5 mmHg (95% CI: -5.7 to -3.2) as well as, with the loss of calories, a reduction in body weight of -1.8 kg (95% CI: -3.5 to -0.1 kg).²³ In patients with renal impairment, the glucose-lowering effects of SGLT2 inhibitors are attenuated.¹⁹ The use of gliflozins is not recommended when glomerular filtration rate (GFR) is under 45 mL/min/1.73 m² (60 mL/min/1.73 m² for dapagliflozin).

Regarding the cardiovascular outcomes, the EMPA-REG OUTCOME trial compared the occurrence of cardiovascular events in 7020 patients with type 2 diabetes and established cardiovascular disease treated by empagliflozin (4687 patients) or placebo (2333 patients). In a mean follow-up time of 3.1 years, empagliflozin was associated with a statistically significant reduction in the primary composite end point of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke by 14% compared to placebo (hazard ratio 0.86, 95% CI: 0.74 to 0.99, p=0.04).²⁴ Deaths from cardiovascular causes were also significantly reduced by 38% in the empagliflozin groups and deaths from any cause by 32%. Published in 2015, these results generated a considerable interest in the medical community. Indeed, such beneficial effects on clinical endpoints have only been observed with antidiabetic drugs in the UK Prospective Diabetes Study (UKPDS) with metformin in 1998²⁵ and, with a lower level of evidence, in a secondary analysis of the PROactive trial with pioglitazone in 2005.²⁶ In the EMPA-REG OUTCOME trial, the relatively low reduction of HbA1c (-0.3% at the end of the trial) suggests that the cardiovascular benefits were not driven by glycemic control. With a 34% reduction in hospitalization for heart failure in the empagliflozin group, the increased osmotic diuresis and natriuresis induced by SGLT2 inhibition may have played an important role, especially in this high cardiovascular risk population including 10% of patients diagnosed with cardiac failure. Nevertheless, the overall findings are not fully explained and could imply other mechanisms such as antiarrhythmic effects, changes in renal function, combination with other cardiovascular drugs (angiotensin converting enzyme inhibitors or angiotensin II receptor blockers,²⁷ increase in glucagon,²⁸ or unknown off-target effects of empagliflozin. Future mechanistic and interaction studies are needed to explore these hypotheses. An on-going study with canagliflozin focused on cardiovascular outcomes (the CANVAS trial) will help in confirming these findings and determining whether or not they are due to a class effect.

Assessment of selectivity

While gliflozins are presented as selective inhibitors of SGLT2, the homology between SGLTs suggests that strict selectivity could not be guaranteed. Table 2 presents the inhibiting potency of SGLT inhibitors according to the different subtypes of SGLT.

Table 2. IC₅₀ of SGLT inhibitors for different subtypes of SGLT and SGLT2 selectivity.^{29,30}

Drug	IC₅₀ (nM) for SGLT2	IC₅₀ (nM) for SGLT1	SGLT2/SGLT1 selectivity*	IC₅₀ (nM) for SGLT4	SGLT2/SGLT4 selectivity*	IC₅₀ (nM) for SGLT5	SGLT2/SGLT5 selectivity*	IC₅₀ (nM) for SGLT6	SGLT2/SGLT6 selectivity*
Dapagliflozin	1.12–1.2	1391–1400	1167–1242	9100	7583	820	683	1300	1083
Canagliflozin	2.7–4.4	684–710	155–263	7900	2926	1700	630	240	89
Empagliflozin	3.1	8300	2677	11000	3548	1100	355	2000	645
Ipragliflozin	5.3–7.38	1876–3000	254–566	16000	3019	740	140	7800	1472
Tofogliflozin	2.9–6.4	8444–12000	1875–2912	14000	2188	3000	469	-	-
Phlorizin	21–34.6	210–290	6-14	6100	290	1500	71	10000	476

*SGLT2/SGLTx selectivity = (IC₅₀ for SGLTx) / (IC₅₀ for SGLT2)

SGLT3 is not represented in this table. It is a glucose-activated ion channel that does not transport sugar.

Table 3. *In vivo* activity of the main gliflozins on SGLT1 in diabetic patients.²⁹⁻³³

Drug	Dosage (mg)	C_{max}* (μM)	IC₅₀ for SGLT1 (μM)	Ratio IC₅₀SGLT1/C_{max}
Empagliflozin	10	0.2	8.3	41.5
	25	0.6		13.8
Dapagliflozin	10	0.5	1.4	2.8
	20	0.7		2.0
Canagliflozin	100	2.5	0.7	0.3
	300	7.8		0.1

*C_{max}: maximum plasma concentration after one single dose in diabetic patients

Gliflozins present various potencies of inhibition of SGLTs other than SGLT2. As always, the degree of inhibition must be valued depending on the effective concentration of the drug at the site of action. For example, in studies where patients were administered 300 mg of canagliflozin before a meal (three times the recommended daily dose), a greater reduction was observed in postprandial glycemia suggesting that high concentrations of canagliflozin in the intestinal lumen could have extensively inhibited SGLT1 in the small intestine and significantly reduced glucose absorption.³⁴ Table 3 presents the activity on SGLT1 of the main gliflozins (for which data were available), based on *in vivo* plasma concentrations observed in patients with type 2 diabetes. Except empagliflozin, the main gliflozins given at commonly used oral dosage were present *in vivo* in plasma concentrations which correspond to a substantial inhibition of SGLT1, especially canagliflozin. Thus, despite the uncertainties regarding the concentrations at the site of action, this suggests that these drugs cannot be considered as strict selective inhibitors of SGLT2 *in vivo*. Thus, *in vivo* inhibition of SGLT1 must be considered as well as, potentially, inhibition of others SGLT (SGLT3 to SGLT6), even though we lack information on their physiological effects.

Potential harmful effects of gliflozins

The harmful effects discussed in the following paragraphs include effects that are currently suspected based on pharmacological hypotheses and for which the evidence is currently relatively scarce. These findings must be interpreted with caution and keeping in mind the patient's individualized benefit/risk ratio.

Infections

Urinary tract infections (UTI) occur in approximately 5% of gliflozin users. In a meta-analysis of gliflozin trials, they were associated with a 42% increase in UTI (OR = 1.42, 95% CI: 1.06 to 1.90).²³ Genital mycotic infections (especially candida infections) such as vaginitis and balanitis occur in 5 to 10% of gliflozin users, predominantly in female. In the same meta-analysis, compared to placebo or other antihyperglycemic agents, OR for genital infections were 3.50 (95% CI: 2.46 to 4.99) and 5.06 (95% CI: 3.44 to 7.45), respectively.²³

The higher risk for urinary tract and genital infection in gliflozin users is explained by the increased and sustained glycosuria that may facilitate the growth of pathogenic microorganisms. In addition, an immunological effect of inhibition of SGLT cannot be excluded since SGLT1 has been suggested to play an immunological role in the protection of sepsis, especially in the gut.³⁵

In the clinical trials, these events were generally mild to moderate, responded to treatment and rarely led to discontinuation of the study drug.³⁶ However, between March 2013 and October 2014, 19 cases of life-threatening urinary tract infections and pyelonephritis were reported to the FDA. All patients were hospitalized and some required admission to intensive care or dialysis.³⁷ Further investigations are needed to assess the magnitude and consequences of the increased risk of urinary and genital infections in real-life populations as well as its long-term effects on renal function and reproduction.

Malignancies

A signal for an increased risk in bladder and breast cancers was raised in dapagliflozin trials and resulted in a delayed commercialization of dapagliflozin in the US in 2011. At this time,

the FDA reported that bladder cancer occurred in 9/5478 patients treated with dapagliflozin, compared with 1/3136 control patients. All 9 dapagliflozin-exposed bladder cancers occurred in males aged from 49 to 75 and within two years of exposure. Although a majority of the cases presented risk factors for bladder cancer, comparison of these risk factors between dapagliflozin and control arms at baseline did not show any imbalance that could have explained numerically increased number of cases with dapagliflozin. Furthermore, bladder cancer incidence observed in males treated by dapagliflozin significantly exceeded the incidence expected in an age-matched reference diabetic population.³⁸ In 2013, the total number of cases reported by the FDA was 10/6045 in the dapagliflozin groups and 1/3512 in control groups, resulting in an incidence rate ratio of 6.11 (CI = 0.827 to 272.02).³⁹ The mechanism that could explain the potential increase risk of bladder cancer with gliiflozins is based on the hypotheses that i) intense and permanent glycosuria could directly promote faster growth of malignant cells and ii) recurrent or chronic gliiflozin-induced urinary tract infections could chronically irritate the bladder epithelium and increase the risk of bladder cancer.⁴⁰

As for the breast cancers, the initial FDA assessment reported that they occurred in 9/4287 patients treated with dapagliflozin, compared with 0/1941 control patients. All dapagliflozin-exposed breast cancers were reported in women aged from 53 to 74 and within less than one year of treatment duration. Similarly, breast cancer risk factors at baseline were similar between the dapagliflozin and control arms. The incidence rate of breast cancer in the dapagliflozin group was higher than those reported in women with type 2 diabetes of the same age.³⁸ In 2013, the FDA reported 3 more cases of breast cancer in dapagliflozin groups and two more cases in control resulting in an incidence rate ratio of 2.47 (95% CI: 0.64 to 14.10).³⁹

There is a possibility that these numerically increased cancer cases could be explained in part by detection bias in the clinical trials by early diagnosis of preexisting cancer. Indeed, the increased incidence of urinary tract infections in the dapagliflozin group may have led to more frequent urine examinations and an early detection of abnormal bladder cytology.⁴¹ Likewise, a similar phenomenon could have promoted the detection of breast tumors in dapagliflozin groups where the increased weight loss was associated with fat reduction in the breasts. Nevertheless, these possible detection errors were not observed in the trials studying other SGLT2 inhibitors.

Experimental data suggest that, in humans, SGLT, especially SGLT1, may play a role in the glucose uptake of malignant cells in various cancers. However, the importance of this role in cancer growth is not well determined.⁴¹ In a study of SGLT2 knockout mice, there were no hyperplasia/neoplasia observed in the urinary bladder mucosa, urogenital tract, or kidneys.⁴² In a two-year carcinogenic animal study, high-dose dapagliflozin was not associated with any signal for mammary or bladder tumor in rats and mice, although atypical hyperplasia of the renal tubules (but no adenoma nor carcinoma) were observed in rats at all doses. Based on the observation that, in the rat, acarbose (an α -glycosidase inhibitor) or other agents that induce glucose malabsorption or imbalanced calcium metabolism are associated with increased tumors in renal tubules, adrenal gland, and testicular Leydig cells, it has been hypothesized that atypical hyperplasia of the renal tubules was linked to inhibition of intestinal SGLT1 by dapagliflozin.^{43,44} In another animal carcinogenicity study, although female rats showed benign and malignant mammary tumors, they were not imbalanced between dapagliflozin and control groups regarding mammary or bladder cancers.⁴² Regarding canagliflozin, long-term carcinogenicity study in rats suggested an increased risk for Leydig cell tumors, renal tubular tumors and pheochromocytoma.⁴⁵ In 2012, there were no reported cases of pheochromocytoma or malignant adrenal tumors in canagliflozin-treated patients. Only one case of testicular cancer

was reported in a patient treated for two months by canagliflozin. It can be hypothesized that inhibition of SGLT1 can play a role in tumorigenicity of adrenal and renal cancer. Indeed, carbohydrate malabsorption results in reduced intestinal pH in the gut lumen and leads to an increased absorption of calcium from food. Abnormal changes in the kidney may be due to mineralization of calcium phosphate crystals in the renal cortex and to excessive calcium excreted by the kidney to maintain calcium homeostasis. This is more probable in rat, where oral bioavailability of canagliflozin is half the one observed in human, providing more canagliflozin in the gut lumen to inhibit SGLT1.³⁴ In clinical studies, there were no report of biological observations such as calcium imbalance or changes in LH or testosterone, furthermore, there were no imbalance for breast, bladder and renal cancer between canagliflozin and control groups.⁴⁵ Regarding empagliflozin, animal studies have shown an increased risk of renal cancer in male mice but not in other animals.⁴⁶ In clinical studies, there were no imbalance for renal or bladder cancer between empagliflozin and control groups. In 2013, the FDA noted a signal for an imbalanced incidence for lung cancer (different cell types) and melanoma in empagliflozin-treated patients (4/2108 patients-years [PY] and 3/7872 PY, respectively) compared to control group (0/1019 PY and 0/4184 PY, respectively). Most of the cases had known risk factors.⁴⁷ It is interesting to note that, in an autopsy study of patient with lung neoplasia, while there was no increased expression of SGLTs in primary lung cancers, SGLT2 was over-expressed in the metastatic lesions of lung cancer.⁴⁸

In conclusion, although causality cannot be established, these safety signals raise concerns about gliflozin-induced cancers and further studies are needed to assess the potential risk of malignancies with the use of SGLT2 inhibitors.

Hepatotoxicity

SGLT2 is expressed in the liver. The predominant mode of gliflozins biotransformation is hepatic metabolism to O-glucuronides. While there were no preclinical signals for hepatotoxicity, in approximately 3000 patients with type 2 diabetes exposed to dapagliflozin in clinical trials, there were five cases of elevated aspartate transaminases (AST) or alanine transaminases (ALT) > 3 times the upper limit of normal (ULN) with total bilirubin > 2 x ULN (this corresponds to biochemical « Hy's Law » criteria). Among them, one case had not an alternative etiology and was classified according to Hy's Law as a « probable » moderately severe dapagliflozin-induced liver injury,³⁸ a signal that has been associated with risk of severe hepatotoxicity potentially resulting in death or transplantation in real-life populations.³⁹ Although subsequent liver biopsy in this suspected case showed signs of autoimmune hepatitis, dapagliflozin-induced liver injury cannot be excluded since no antibodies were detected.⁴⁹ In canagliflozin trials, two possible cases of drug-induced liver injury were detected, but complete clinical data were lacking.⁴⁵ As for empagliflozin, cases fulfilling biochemical Hy's Law criteria were numerically increased in empagliflozin groups in clinical trials. However, all these cases appeared to have another possible etiology of liver injury.⁴⁷

In conclusion, drug-induced liver injury cannot be excluded with gliflozins. Although an elevated hepatotoxicity seems not probable, evaluation of their hepatic safety in real-life populations must be continued.

Fasting hypoglycemia

As the glucose-lowering effect of gliflozins neither involves increased insulin secretion nor inhibits hormonal regulation resulting in glucose production secondary to hypoglycemia,⁵⁰ fasting hypoglycemia is not an adverse reaction that is expected by inhibition of SGLT2. Indeed, hypoglycemia is rarely seen in patients with SGLT2 gene defect in familial renal glycosuria.⁵ Although SGLT2 knockout mice do not developed hypoglycemia, they showed substantially increased caloric intake that could be secondary to minor unnoticed hypoglycemia.⁵¹ In clinical trials, severe hypoglycemia was rare in gliflozin groups but the risk of hypoglycemia was increased in patients co-administered with a sulfonylurea or insulin.^{19,34} A meta-analysis of clinical trials indicated that, compared with placebo, OR for hypoglycemia events with gliflozins was 1.28 (95% CI: 0.99 to 1.65) and that, compared with other antihyperglycemic agents including sulfonylureas, OR was 0.44 (95% CI: 0.35 to 0.54).²³ The minimal risk of hypoglycemia with gliflozins could be due to less inhibition of SGLT1 transporters that may reabsorb sufficient amount of sugar from the urine to avoid severe hypoglycemia. However, these data have to be confirmed in real-life conditions and already encourage close monitoring of hypoglycemia in gliflozins-treated patients and, if necessary, dosage adaptation of add-on insulin-secreting drugs.

Hypovolemic effects

Gliflozin-induced glycosuria is associated with significantly increased diuresis (approximately +375 mL/day). As a consequence, volume depletion-related adverse events occurred in approximately 1% to 3% in clinical trials.⁵⁰ These events include dehydration, hypovolemia, but mostly hypotension or orthostatic hypotension. Compared to control groups, hypotension was more frequent in gliflozin-treated groups, especially for patients with risk factors such as advanced age, moderate renal impairment, or treatment with loop diuretics.^{39,45} The diuretic effect of SGLT2 inhibitors induces a persistent increase in hematocrit (approximately +2%).^{24,39} Hemoconcentration may increase the risk of venous thromboembolism but such events were not considered as a substantial concern in clinical trials: they were often not serious and occurred in similar rate among groups (approximately 0.3%), or were not reported.^{34,49} In conclusion, hypovolemic events are frequent with gliflozins. Additional data are needed before firm conclusions can be drawn about serious consequences of hemoconcentration.

Ketoacidosis

Ketoacidosis is an excessive acidity of the blood caused by the accumulation of ketone bodies such as 3-hydroxybutyric acid and acetoacetic acid. Ketones are byproducts of fatty acid oxidation and can be used as energy substrates, especially in the brain. They are produced when the lack of intracellular glucose raises glucagon levels, increases delivery of free fatty acids (lipolysis), and stimulates hepatic catabolism of fatty acids at the expense of carbohydrate oxidation. Ketoacidosis is generally observed in patients with type 1 diabetes as an inaugural diagnosis of the disease or secondary to infection, surgery or other conditions that demand increase amount of glucose (it is enhanced by concomitant use of steroids or betamimetics). In insulin-lacking patients, ketoacidosis is generally associated with severe hyperglycemia, glycosuria and ketonuria that cause dehydration potentially resulting in fatal shock.

As of May 2015, the FDA reported 73 post-marketing cases of ketoacidosis in patients treated with gliflozins for type 2 and type 1 diabetes (the last is an off-label use).³⁷ In Europe, 102 similar cases were recorded in Eudravigilance database.⁵² Unlike the usually described

diabetic ketoacidosis, these cases were associated with moderately or no elevated blood glucose levels (they were called “euglycemic”). This characteristic is likely to lead to delays in diagnosis and treatment.⁵³ Some of the ketoacidosis cases were triggered by factors such as infections, renal impairment, kidney damage, trauma, reduced food and fluid intake, hypoxemia, abuse of alcohol and lower doses of insulin.³⁷ In preclinical studies and clinical trials, mild to modest increases in ketone concentrations were measured.⁵² Retrospectively analyzed, the frequency of ketoacidosis reported in clinical trials of gliflozins was less than 0.1%.⁵²

Euglycemic ketoacidoses have already been described in patients with diabetes (principally type 1).^{54,55} However, several mechanisms can contribute to explain a specific risk of gliflozin-induced ketoacidosis. First, the use of SGLT2 inhibitors induces a reduction of glycemic level that, in response, leads to decreased insulin levels and improved insulin sensitivity. Surprisingly, SGLT2 inhibitors are also associated with a significant increase in hepatic glucose production and fasting plasma glucagon concentrations.^{56,57} The hyperglucagonemia can be explained by i) the decreased levels of insulin which, in the pancreatic islets, exerts an inhibitory effect on glucagon release⁵⁸ and/or ii) the direct inhibition of SGLT2 transporter which, located in the membrane of alpha cells, is thought to play a role in glucose uptake triggering glucagon production.⁵⁹ In any cases, the gliflozin-induced hyperglucagonemia may be responsible for a higher hepatic ketone production leading to ketoacidosis, especially when glycogen stores are depleted (fasting or low carbohydrate intake).⁵² Second, SGLT2 inhibitors are associated with significant volume depletion, which could contribute to aggravate the symptoms of ketoacidosis. Third, SGLT2 inhibitors are associated with increased risk of infections which are known triggering factors of ketoacidosis. Fourth, in the most severe type of familial renal glycosuria (type 0), there is a complete absence of tubular glucose resorption and several patients have developed dehydration and ketosis during pregnancy or starvation state.⁶⁰ Fifth, it has been hypothesized that SGLT2 inhibitors could also increase the tubular reabsorption of ketones and worsen the plasma ketones levels by reducing their renal elimination.⁶¹ Indeed, a study has shown that phlorizin, the historical non-selective SGLT1/SGLT2 inhibitor, promotes tubular reabsorption of acetoacetate in dogs.⁶² In physiological conditions, ketones are cotransported with Na⁺, from the tubular lumen into the tubular epithelial cells, according to the sodium concentration gradient. SGLT inhibition, by increasing Na⁺ concentration in the tubular lumen, reinforces the sodium concentration gradient and potentially accentuates ketones reabsorption.⁶¹ In addition, ketonuria being an early sign of ketoacidosis, increased ketones reabsorption could delay or mislead its diagnosis. Finally, in clinical trials, empagliflozin-treated patients were more likely to have significant low serum bicarbonate.⁴⁷ This effect could theoretically increase the risk of acidosis.

In conclusion, ketoacidoses observed in gliflozin-treated patients differ from usually described diabetic ketoacidoses by the intense glycosuria that can lower plasma glucose to almost normal levels and by potential side effects that can stimulate ketones production, inhibit ketones elimination or aggravate the symptoms of ketoacidosis.^{52,63} Clinicians must be even more aware of the potential risk of diabetic ketoacidosis in gliflozin-treated patients than its presentation can be uncommon and makes its diagnosis more difficult.

Effects on bone

In a long-term study in patients with moderate renal impairment over a period of two years, bone fracture occurred in 13/168 patients treated by dapagliflozin compared to 0/84 in the placebo group.⁶⁴ In canagliflozin trials of 60-70 weeks, overall incidences of bone fracture

were 18.1 and 14.2 per 1000 PY for canagliflozin and comparator groups, respectively (no imbalance was observed in patients with renal impairment).⁴⁵ In the CANVAS study (where patients had a history or risk of cardiovascular disease), a significant increase in bone fractures was observed with canagliflozin (4.0%) vs. placebo (2.6%).⁶⁵

In rat toxicology studies, increased urinary calcium, hyperostosis (increase in trabecular bone volume), reduced levels of parathormone (PTH), 1,25-OH vitamin D and bone turnover markers were observed with both canagliflozin and dapagliflozin. Although the mechanisms are not fully understood, these findings could be secondary to a hyperabsorption of calcium due the inhibition of intestinal SGLT1. The fermentation of unabsorbed sugars in the gut lumen could lower the intestinal pH, and results in increased absorption of dietary calcium.^{38,45} In contrast, in clinical trials, gliflozins were not associated with changes in calcium levels but increased phosphatemia, probably via an increased tubular reabsorption of phosphate. Indeed, the inhibition of SGLT2 increased the intraluminal concentration of Na⁺ which results in augmented cotransport of phosphate and Na⁺ from the tubular lumen into epithelial cells.⁶⁶ Hyperphosphatemia induces the liberation of PTH which is an important factor of bone resorption. A transient increase in PTH levels was noted in clinical trials of both canagliflozin and dapagliflozin. Canagliflozin has also been associated with a slight reduction of 1,25-OH vitamin D, which can result in decreased bone formation. Decrease in 1,25-OH vitamin D is contradictory with elevated PTH, but it can be explained by the potential effect of fibroblast growth factor 23 (FGF23) stimulated by increased phosphatemia.⁶⁶ The use of canagliflozin was also associated with a slight increase in magnesemia, increases in bone resorption biomarkers, and reductions in bone mineral density in the total hip after two years.^{45,67,68} While the biological changes (such as PTH and 1,25-OH vitamin D) observed with canagliflozin were small, the large variability among patients indicates that, in some patients, SGLT2 inhibitors could substantially affect bone metabolism and increase the risk of fracture.⁶⁶

The potential increase in bone resorption could also be related to significant gliflozin-induced weight loss especially when the weight loss is not associated with exercise.⁴⁵ In addition, gliflozins could also have an influence on the risk of fall. Indeed, fractures observed with canagliflozin generally concerned extremities of the upper limbs (which is not usual in bone fragility). Furthermore, some fractures occurred within 12-26 weeks, and were associated with events such as hypoglycemia or orthostatic hypotension.⁴⁵

In conclusion, the risk of fracture with gliflozins probably depends on a combination of factors that needs to be further examined. Nevertheless, if this risk has mainly been highlighted for canagliflozin, a class effect on bone safety cannot be excluded without conclusive long-term studies.

Effects on kidney function

In non-clinical studies, exposure to gliflozins at two to five times clinical doses induced mineralization of tubules and papillae and dilatation of the renal pelvis, ureter and bladder of mice and rats. These findings were considered as minor consequences of chronic glycosuria, polyuria and calciuria. At higher doses (more than 10 to 47 times clinical doses), the nephrotoxicity observed in the different species included tubular karyomegaly, cell necrosis, cystic hyperplasia or hypertrophy, renal mineralization, tubular nephropathy and interstitial nephritis.⁴⁷ In clinical studies, increase in serum creatinine and reductions from baseline in estimated glomerular filtration rate (GFR), up to 30%, were seen in 1-4% of gliflozin-treated patients, more frequently than in control groups. The changes in markers of renal impairment

were generally dose-dependent, moderate, early (during the first few weeks of treatment) and transient (return to baseline in less than 24 weeks). They were much larger in patients with moderate renal impairment (GFR < 60 ml/min/1.73 m²), older patients (age ≥ 65) or patients using diuretics, angiotensin converting enzyme inhibitors or angiotensin II receptor blockers.^{38,46} In addition to these changes, hyperkalemia was also observed with canagliflozin in clinical trials.⁴⁵

Patients with type 2 diabetes frequently present with glomerular hyperfiltration (defined by a GFR > 135 ml/min/1.73 m²) as an initial stage of diabetic nephropathy. A hypothesis for this increased GFR has involved a role of SGLT in the mechanism of tubuloglomerular feedback.⁶⁹ The diabetic state could induce an increased activity and number of SGLT2 and therefore an enhanced reabsorption of Na⁺ in the proximal tubule. The resulting water retention is thought to be one of the mechanisms responsible for hypertension in diabetic patients.⁷⁰ Thereby, SGLT2 inhibitors, providing an increased amount of Na⁺ at the macula densa in the distal convoluted tubule, induce (by activation of the tubuloglomerular feedback) a vasoconstriction of the afferent arterioles and reduce the GFR. Indeed, attenuation of renal hyperfiltration has been demonstrated with empagliflozin in patients with type 1 diabetes.⁷¹ No effects were seen in patients with normal GFR. Hence, by restoring an adequate GFR in patient with glomerular hyperfiltration, the role of SGLT2 inhibitors on kidney function could appear as a favorable nephroprotective effect. However, it is hypothesized that increased glycosuria (and albuminuria) produced in the proximal tubule could cause damages to the downstream nephron, which could be sensitive to glucotoxicity.⁷² The renal consequences of long-term exposure to SGLT2 inhibitors remain to be established.

In conclusion, due to the expected decrease in GFR, regular monitoring of renal function of gliflozin-treated patients should be performed. It should be even more careful in elderly, in patient with renal impairment, hypovolemia or additional combination of drug decreasing blood pressure, drugs decreasing GFR (e.g. diuretics or nonsteroidal anti-inflammatory drugs) or drugs which induce toxic effects when their renal elimination is decreased (e.g. metformin). It remains to stay vigilant as more research is needed to determine the long-term renal effects of gliflozins.

Lipid changes

Increased lipoproteins (both high and low-density, HDL and LDL) and total cholesterol were observed with gliflozins. The most notable effect was a marked dose-dependent increase in LDL-C levels induced by canagliflozin. Compared to placebo, the mean increase in LDL-C levels was 8.2 mg/dL (0.2 mmol/L) (+12% in one canagliflozin trial). These changes were not affected by age, sex, race, body mass index, estimated GFR, baseline statin use, and baseline LDL-C.⁴⁵ Increase in LDL-C was also observed with dapagliflozin and empagliflozin.^{39,47}

Although it is not well understood, gliflozin-induced increase in serum lipoproteins could be due to several factors including hemoconcentration, increased food consumption, or a potential consequence of the increased diuresis. Regarding the latter, it has been suggested that high-dose thiazides are associated with a short-term increase in total cholesterol (+4%) and LDL-C levels (+10%). However, this effect is controversial and the mechanism not fully explained.⁷³ As for the increased food intake, although it was not clearly measured in clinical trials, hyperphagia has been established as a compensatory mechanism of gliflozin-induced weight loss in obese rats.⁷⁴

Some findings tend to limit the clinical impact of the increased LDL-C. First, gliflozins are associated with a larger increase in the large LDL-C subfraction, which is the less atherogenic.

Second, a concomitant increase in HDL-C results in almost unchanged LDL-C to HDL-C ratio.³⁴ Nevertheless, the safety of gliflozins regarding atheromatic events is not established and the potential clinical consequences of these lipids changes have to be determined.

Cardiovascular safety

Contrary to other studied species (rats, dogs, pigs or monkeys), SGLT1 carriers are found in the human cardiac tissue and are thought to be significantly upregulated in patients with type 2 diabetes.⁷⁵⁻⁷⁷ The clinical consequences of the potential inhibition of SGLT1 in the heart, especially in the long term, are unknown.

In accordance with the favorable changes in HDL-C, blood pressure, and body weight, the EMPA-REG OUTCOME showed a decreased cardiovascular mortality with empagliflozin.²⁴ In a meta-analysis of clinical trials with all gliflozins including regulatory submissions (37,525 patients) and published trials (33,385 patients), beneficial effects were demonstrated on major adverse cardiovascular events, cardiovascular death, heart failure, and all-cause mortality.⁷⁸ However, non-fatal stroke increased by 30% and no beneficial effects were found for myocardial infarction or angina. These findings raise the concern that gliflozins may be associated with a risk of arterial thrombosis that could be explained, at least in part, by hemoconcentration and/or changes in lipids.

In canagliflozin trials which involved patients at high cardiovascular risk, a slight and not statistically significant increase in lower limb amputations was recently noted by the European Medicine Agency (5 to 7 in 1000 PY with canagliflozin versus 3 with placebo).⁷⁹ Although this signal has yet to be confirmed, it is interesting to note that arterial thrombosis, increased risk of infection, decreased blood pressure which influences the vascularization of extremities are at the same time potential effects of gliflozin and risk factor for amputation.

Despite favorable changes in cardiovascular events, especially heart failure, observed in patients treated with gliflozins, some questions regarding their cardiovascular safety and particularly the risk of arterial thrombosis remain to be elucidated.

Intestinal effects

In the duodenum and jejunum, resorption of glucose is mediated by SGLT1, which cotransports glucose and galactose with Na⁺. As it is observed in genetic defects of SGLT1, drug-induced inhibition of SGLT1 would translate into various degrees of carbohydrate malabsorption. Indeed, diarrhea and dehydration were important adverse reactions of phlorizin.¹⁶ Gliflozins show a lesser inhibitory potency over SGLT1 than phlorizin (Table 2). However, given the uncertain *in vivo* selectivity of gliflozins and the fact that, orally administered, they remain present in the intestine at relatively high concentrations before being absorbed, they could exert a relevant inhibitory effect on intestinal SGLT1, even at usual doses. However, this theoretical mechanism did not translate into clinical observations. In clinical trials, incidence of glucose-galactose malabsorption symptoms such as diarrhea or flatulence was low and similar to control groups.

Effects in pregnancy and lactation

Gliflozins have not been investigated in pregnant or breastfeeding women as they were excluded from all clinical studies. Nevertheless, in clinical trials, four patients became pregnant while being treated with empagliflozin. Two pregnant women exposed during 2 and 217 days gave birth to healthy babies. The two other exposed 22 and 33 days had an abortion and a miscarriage, respectively.⁴⁶ In preclinical studies, gliflozins were detected in fetal tissue and breast milk. No impact was noted on fertility, implantation, or fetal development. Studies on juvenile rats demonstrated that gliflozins caused abnormalities in renal development and maturation. Rat exposed to high-dose dapagliflozin from birth to approximately 13 weeks of age (a period of morphological and functional development of the kidneys) showed dilatation of the renal pelvis and tubules and inhibited weight gain. These findings were thought to be a consequence of the gliflozin-induced glycosuria and polyuria in an immature kidney and could not be excluded at doses near the clinical doses.^{44,46} Hence, it is prudent to contraindicate the use of gliflozins during pregnancy and lactation.

Conclusions

Despite favorable effects on glucose level, blood pressure and promising findings on cardiovascular mortality observed with gliflozins, the enthusiasm for this new class of drugs must be tempered by the relatively short delay since their marketing and by the uncertainties regarding the safety points discussed in this article. Given that *in vivo* selectivity to SGLT2 is not guaranteed and may vary between specific drugs, it remains to be determined whether long-term use of gliflozins produces effect in organs where other SGLTs are expressed (e.g. brain, lung, muscle, pancreas, prostate, etc.). Other organs and functions could also be affected by sustained metabolic changes induced by gliflozins. Hence, long-term studies in large populations of patients with type 2 diabetes are needed to better assess the benefits and risks of gliflozins and to determine the effects that apply to the whole class and those which are drug specific.

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