ABSTRACT

Remarkable progress has been achieved in the field of diabetes with the development of incretin analogues, dipeptidyl peptidase IV inhibitors and novel insulin analogues; nevertheless, there is an unmet need for additional therapeutic options. Individualization of HbA1c target levels is a recent progress within the field. Approximately 50% of diabetics do not reach a previously aspired treatment goal of glycosylated HbA1 levels below 7% and often face a vicious circle with accelerated weight gain. Current antidiabetic therapeutics mainly target the decline in insulin secretion and ameliorate insulin resistance. In this regard a new generation of drugs, denoted gliptozines, that specifically interfere with sodium-glucose cotransporters (SGLT)-2 and exhibit a favourable impact on glucose metabolism in patients with type 2 diabetes are emerging as hopeful avenues. The resultant negative energy balance caused by glucosuria results in long-term weight losses, significantly reduced HbA1c levels approximating 0.5–1.0% and may in addition exert beneficial effects on blood pressure, reactive oxygen products and inflammatory mediators. Recent studies indicate improvement in β-cell glucose sensitivity and insulin sensitivity in patients treated with gliptozines, a decrease in tissue glucose disposal and interestingly an increase in endogenous glucose production. The list of side effects observed under SGLT2 inhibition includes increased rates of genital infections, balanitis, vulvovaginitis, hypotensive episodes and acute deterioration of kidney function. Main questions towards the safety profile are still unanswered given that long-term clinical outcome data with SGLT2 inhibition are lacking and the cardiovascular safety profile is under scrutiny in large trials. Thus, the successful development of selective SGLT2 inhibitors for therapeutic use in diabetics has a huge potential to meet patients’ needs. However, it awaits quick results from clinical trials with meaningful clinical endpoints.

Keywords: diabetes, gliptozines, glucosuria, inflammation, nephropathy

GLUCOSE METABOLISM IN THE KIDNEY

The kidney is a key player in glucose metabolism. Under physiological conditions, the kidney filters ~180 L of plasma per day [1, 2]. At ~90–100 mg/dL plasma glucose levels, almost all glucose (~162–180 g) is filtered and reabsorbed primarily in the proximal tubule and returned to the circulation [1, 2]. At glucose concentrations exceeding a ‘threshold’ of 160–180 mg/dL, the maximum capacity of glucose transport (Tm) of the proximal tubule (on average ~375 mg/min) is exceeded, resulting in excretion of glucose in the urine [1, 2].

Renal glucose reabsorption in the proximal tubule is regulated by sodium-glucose transporters (SGLTs) and facilitative glucose transporters (GLUTs) [1, 2] (Figure 1). GLUTs facilitate glucose movement from the extracellular to the intracellular space along its chemical gradient without energy consumption, while SGLTs actively transport glucose against its concentration gradient using the energy provided by sodium cotransport [3–5].

Seven different SGLTs have been identified so far [6]; SGLT2 and SGLT1 are best characterized in humans and have a defined role in renal glucose reabsorption [5, 6]. The human SGLT1 transporter is expressed in the intestinal mucosa transporting glucose and galactose across the intestinal mucosa. In the kidney, it is expressed in the S3 segment of the proximal tubule.
tubule and is responsible for ~10% of glucose reabsorption [1, 7]. Human SGLT2 is primarily expressed in the S1 segment of the proximal tubule. It is a low-affinity and high-capacity transporter responsible for ~90% of glucose reabsorption [1, 8].

In the light of the evolutionary process, increased renal SGLT2 expression in response to high plasma glucose concentrations, as observed in experimental settings [9], may contribute to conservation of energy and particularly glucose for the brain. However, in patients with type 2 diabetes and increased Tm for glucose [10], this mechanism increases hyperglycaemia. Therefore, efforts have been undertaken to block renal glucose reabsorption leading to the development of SGLT2 inhibitors.

**HISTORICAL OVERVIEW AND DEVELOPMENT OF SGLT2 INHIBITORS**

The first model for active transport of glucose and other molecules was proposed by Bob Crane in 1960 at the Symposium on Membrane Transport and Metabolism in Prague [11], where he described the Na⁺/glucose cotransport hypothesis. According to Crane’s hypothesis, glucose is actively transported across the plasma membrane by a Na⁺/glucose cotransporter driven by the inward Na⁺ gradient maintained by the Na⁺ pump. This process could be blocked directly by phlorizin-mediated inhibition of cotransport or indirectly through strophantidin-mediated inhibition of the Na⁺ pump and associated reduction of the Na⁺ gradient (discussed in [6]).

Experimental data in support of the presence of higher- and lower-affinity Na⁺/glucose transporters at different renal sites [12–14], as well as the absence of clinical overlap in inherited defects of glucose transport in the kidney (such as familial renal glucosuria) or intestine (such as intestinal glucose/galactose malabsorption), suggested the presence of at least two different Na⁺/glucose transporters and ultimately led to the cloning and characterization of SGLT1 (chromosome 22 q13.1) and SGLT2 (chromosome 13 p11.2) [15–17].

Phlorizin was isolated from the bark of apple trees in 1835 by the French chemist Petersen with first reports on its glucosuric action [2]. It was the first SGLT inhibitor to be identified [18, 19]. Phlorizin is a 2’-[glucoside of phloretin, belonging to a group of dihydrochalcones, a type of flavonoids [2] and inhibits both SGLTs in the proximal tubule with a 6-fold higher affinity for SGLT2 than SGLT1. The main disadvantages of phlorizin are its limited bioavailability (~15%) and adverse gastrointestinal (GI) effects [1, 2].

The first orally available derivative of phlorizin, T-1095, was developed in 1999 [20]. However, due to lack of selectivity and safety concerns, further development was discontinued [1, 2]. Other SGLT inhibitors developed at an early stage include sergiliflozin and remogliflozin [1, 2], which showed SGLT2 selectivity and promising glucosuric effects in patients with type 2 diabetes [2, 21]. Atigliflozin [22] and TS-033 [23] also displayed SGLT2 selectivity and reached phase II clinical trials [2] but further development was discontinued. Despite initial promising results, the development of these O-glucoside compounds was discontinued at an early stage, probably because of hydrolytic susceptibility to β-glucosidase degradation and associated gastrointestinal adverse effects [2]. The discovery of C-glucoside derivatives, such as canagliflozin and dapagliflozin, enabled bypass of glucosidase susceptibility and degradation, thus reducing gastrointestinal side effects of these compounds. At present, at least eight SGLT2 inhibitors with differing characteristics have been developed and are being tested in clinical trials, selectivity ranges from 155- to 2912-fold for SGLT2 over SGLT1 [24].

**SODIUM-GLUCOSE COTRANSPORTER 2 INHIBITION: EFFECTS IN EXPERIMENTAL DIABETES MODELS**

Approximately 60% of filtered glucose is lost into the urine of animals with genetic deficiency of SGLT2. Experimental evidence have been collected to determine whether dapagliflozin acts in a nephroprotective manner in diabetes. To this extent experimental models have been set up in rodents. In db/db mice, dapagliflozin applied for 12 weeks markedly reduced the inflammatory response in the kidney, such as less macrophage infiltrates, lower cytokine levels (e.g. TGF-β and MCP-1) as well as lower apoptosis rates. These alterations were recapitulated in an *in vitro* tubular cell model [25]. Most importantly, extensive experimental studies indicate anti-oxidative effects by SGLT2 inhibition, that is suppressed high-glucose induced ROS generation, with dapagliflozin. Such protective effects were also seen in a type 2 diabetic rat model [25].

**SODIUM-GLUCOSE COTRANSPORTER 2 INHIBITION: EFFECTS BEYOND GLUCOSURIA**

In the April issue of the Journal of Clinical Investigation, Ferrannini and colleagues report the metabolic response to SGLT2 inhibition in type 2 diabetic patients [26]. Sixty-six patients with type 2 diabetes were evaluated at baseline, after a
single 25 mg empagliflozin dose (acute study), and after a 4-week daily treatment (chronic study).

Every time the patients received a mixed meal coupled with dual-tracer glucose, enabling the quantification of the separate contribution of meal-derived glucose, endogenous glucose production (EGP) and whole-body glucose disposal to plasma glucose concentrations. With the use of indirect calorimetry, changes in substrate utilization and energy expenditure were assessed.

**Acute study**

A single dose of 25 mg empagliflozin induced glucosuria during fasting (3 h preceding meal ingestion) and after meal ingestion. Fasting plasma glucose dropped and plasma insulin decreased more than glucagon, resulting in a decreased prehepatic molar concentration ratio. After meal ingestion, glucose and insulin decreased, while the glucagon response increased. Fasting EGP was increased ∼30%, and EGP was also greater throughout meal absorption. Oral glucose appearance was unchanged, but tissue glucose disposal (TGD) was reduced (20%) due to decreased non-oxidative glucose disposal, despite an improvement in insulin sensitivity. No changes were observed in lipid or protein oxidation, resting energy expenditure or meal-induced thermogenesis. Empagliflozin at 25 mg increased glucose sensitivity and decreased total insulin output, enhanced the GLP-1 response to the meal and blunted free fatty acid (FFA) suppression.

**Chronic study**

Administration of 25 mg empagliflozin daily for 4 weeks also induced fasting and post-prandial glucosuria and decreased HbA1c and plasma glucose. Similar to the results obtained in the acute study, empagliflozin increased EGP, decreased fasting and post-prandial plasma insulin levels and the glucagon response was still increased, but somewhat less than in the acute study. Fasting and post-prandial TGD was also decreased, with both glucose oxidation and non-oxidative glucose disposal contributing to the decrease. There was a concomitant rise in lipid oxidation, while no changes in protein oxidation or meal-related energy expenditure were observed. The effects on β-cell glucose and insulin sensitivity, GLP-1 response and FFA suppression were similar to those in the acute study.

**GLIFLOZINES IN THE CLINICAL SETTING**

**Canagliflozin**

The C-glucoside canagliflozin (Invokana) was approved by the US Food and Drug Administration (US FDA) in 2013 (once daily 100 or 300 mg tablet) for the treatment of type 2 diabetes [2, 27]. Appropriate dosing depends on renal function: at an estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73 m² the starting dose is 100 mg and can be increased to 300 mg once daily. At an eGFR ≤ 60 mL/min/1.73 m² the recommended dose is 100 mg daily, while at an eGFR < 45 mL/min/1.73 m² canagliflozin is not recommended [2, 27]. Treatment with canagliflozin as monotherapy or in combination with other oral agents such as metformin, sulfonylurea and pioglitazone or with insulin results in significant reduction in HbA1c and fasting plasma glucose levels [1, 2]. Adverse side effects include genital mucotic and urinary tract infections, polyuria with associated reduction of intravascular volume and hypotension, increased low-density lipoprotein cholesterol, increased serum creatinine levels and decreased eGFR (usually transient), electrolyte disorders (such as hyperkalaemia, hypermagnesemia and hyperphosphatemia) and a slightly higher incidence of fracture compared with placebo [1, 2].

Treatment is contraindicated in patients with an eGFR < 45 mL/min/1.73 m², end-stage renal disease undergoing renal replacement therapy, furthermore prescription for children and adolescent at age <18 years is not recommended, pregnancy and lactation are contraindications [2].

**Dapagliflozin**

The C-glucoside dapagliflozin (Forxiga) was approved by the European Medicines Agency (EMA) in April 2012 and by the US FDA in January 2014 (once daily 5 or 10 mg tablet) for the treatment of patients with type 2 diabetes [1, 2, 28]. It is not recommended for patients with an eGFR < 60 mL/min/1.73 m².

Completed phase III trials show that treatment with dapagliflozin (5 or 10 mg daily) consistently decreases HbA1c (>0.5%) compared with placebo. Reductions in fasting and post-prandial plasma glucose contributed equally to the reductions in HbA1c [1]. In addition to monotherapy, dapagliflozin is also recommended in combination with metformin, insulin and insulin sensitizers [2]. Reductions in HbA1c were independent of the duration of diabetes: Zhang et al. [29] treated 151 patients with new-onset diabetes and 58 patients with long-standing diabetes and obtained comparable reductions in HbA1c.

Adverse effects include increased risk of vulvovaginitis and balanitis, probably associated to dapagliflozin-associated glucosuria and dehydration-associated hypotension [28]. As an increased number of bladder cancers were diagnosed among dapagliflozin users in the clinical trials, the use is not recommended for patients with active bladder cancer [28].

Other C-glucosides in late stages of clinical development include empagliflozin and ipragliflozin, which both cause dose-dependent glucosuria, decrease HbA1c and fasting plasma glucose, as well as body weight [1, 30–33].
In one trial the relative risk of bladder and breast cancer was increased in a dapagliflozin treatment group, resulting in a transient hold signal of the FDA advisory committee board. Given that additional clinical data with 8000 person-years exposure did not show any differences in cancer incidence, the approval by the FDA was thereafter provided in 2014 [24]. In patients suffering from bladder or breast cancer, gliflozines should not be prescribed until more clinical data are available.

One of the major issues still under debate is the cardiovascular risk profile of SGLT2 inhibition. The incidence of cardiovascular events was observed to be increased in the first 30 days post-initiation of treatment with a hazard ratio of 6.5 (95% CI 0.85–49.66), likely due to volume depletion and hypotensive episodes [24]. Similarly, stroke may be more often in patients undergoing hypotensive episodes. A large prospective randomized double-blinded placebo-controlled study with recruitment of 17 000 adult type 2 diabetes patients has been initiated by AstraZeneca/Bristol-Myers Squibb to determine the effect of dapagliflozin on cardiovascular outcome as add-on therapy to current treatment standards (DECLARE study). Expected completion is in 2019. Similar studies are ongoing that test canagliflozin and empagliflozin (NCT-01032629, NCT-01131676, NCT-01730534).

Transient hypotensive episodes due to osmotic diuresis are the most likely causes for renal-related adverse events, such as acute kidney injury [34].

The use of SGLT2 inhibition with moderate impairment of kidney function is not recommended, although a recent study indicates that despite abrogated improvement of hyperglycaemia there is still a reduction in body weight and blood pressure [35]. According to the FDA recommendation, dapagliflozin should not be used in diabetics with ketoacidosis and is contraindicated in those with moderate-to-severe renal impairment or dialysis. Nonetheless more clinical data are urgently needed. The mild diuretic effect with an excess diuresis of 200–400 mL/day in the first 2–3 days following initiation of therapy is not accompanied by electrolyte disorders. The reported experimental data suggest anti-oxidant and anti-inflammatory effect of gliflozines, which may outweigh the transient rise of creatinine values due to volume contraction with renal impairment. In a 52-week efficacy and safety study of type 2 diabetes patients with chronic kidney disease stage 3 ($n = 269$, mean eGFR 39.4 mL/min), canagliflozin was well tolerated and even reduced urine albumin to creatinine ratios versus placebo by 28% [36].

**OUTLOOK**

The novel mode of action of gliflozines resulting in a negative energy balance with weight loss or neutral weight development as well as positive effects on whole-body metabolic effects renders SGLT2 inhibition attractive.

Future studies are needed to address the potential synergistic effects of pharmacotherapy with SGLT2 inhibitors in combination with other antidiabetics and will address safety issues that relate to cardiovascular outcome, cancerogenesis as well as side effects on kidney function. Experimental data suggest that gliflozines may provide beneficial effects for patients with incipient diabetic nephropathy. If confirmed in future studies, this would render them even more appealing, especially for kidney patients.

**CONFLICT OF INTEREST STATEMENT**

The results presented in this paper have not been published previously in whole or part.

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Kidney paired donation: principles, protocols and programs

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ABSTRACT

Due to the ongoing shortage of deceased-donor organs, novel strategies to augment kidney transplantation rates through expanded living donation strategies have become essential. These include desensitization in antibody-incompatible transplants and kidney paired donation (KPD) programs. KPD enables kidney transplant candidates with willing but incompatible living donors to join a registry of other incompatible pairs in order to find potentially compatible transplant solutions. Given the significant immunologic barriers with fewer donor options, single-center or small KPD programs may be less successful in transplanting the more sensitized patients; the optimal solution for the difficult-to-match patient is access to more potential donors and large multicenter or national registries are essential. Multicenter KPD programs have become common in the last decade, and now represent one of the most promising opportunities to improve transplant rates.

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