SGLT Inhibitors as New Therapeutic Tools in the Treatment of Diabetes

Rolf K.H. Kinne and Francisco Castaneda

Contents

Abstract Recently, the idea has been developed to lower blood glucose levels in diabetes by inhibiting sugar reabsorption in the kidney. The main target is thereby the early proximal tubule where secondary active transport of the sugar is mediated by the sodium-D-glucose cotransporter SGLT2. A model substance for the inhibitors is the O-glucoside phlorizin which inhibits transport competitively. Its binding to the transporter involves at least two different domains: an aglucone binding site at the transporter surface, involving extramembranous loops, and the sugar binding/translocation site buried in a hydrophilic pocket of the transporter. The properties of

R.K.H. Kinne (✉) and F. Castaneda
Max-Planck-Institute of Molecular Physiology, Otto-Hahn-Str. 11, 44227 Dortmund, Germany
e-mail: rolf.kinne@mpi-dortmund.mpg.de

M. Schwanstecher (ed.), *Diabetes - Perspectives in Drug Therapy*,
Handbook of Experimental Pharmacology 203,
DOI 10.1007/978-3-642-17214-4_5, © Springer-Verlag Berlin Heidelberg 2011

these binding sites differ between SGLT2 and SGLT1, which mediates sugar absorption in the intestine. Various O-, C-, N- and S-glucosides have been synthesized with high affinity and high specificity for SGLT2. Some of these glucosides are in clinical trials and have been proven to successfully increase urinary glucose excretion and to decrease blood sugar levels without the danger of hypoglycaemia during fasting in type 2 diabetes.

Keywords Blood glucose control · Diabetes · Glucosides · Renal sugar transport · Sodium-D-glucose cotransporter

1 Preface

One of the main features of diabetes is the elevation of blood sugar with its deleterious consequences in a variety of tissues (Ceriello 2005). Thus, control of the plasma glucose level is of utmost importance in the treatment of this disease. In recent years, the idea has evolved that affecting glucose absorption in the intestine and/or the glucose reabsorption in the kidney might be a possible way to control the sugar level. Therefore, initially inhibitors of sugar absorption have been developed which inhibit the hydrolysis of sucrose and lactose by disaccharidases in the intestinal lumen. Examples that have been successfully introduced in the market are acarbose (Precose R or Glucobay R), voglibose (Basen R) and miglitol (Glyset R) (Asano 2003; de Melo et al. 2006). As the molecular understanding of sugar transport progressed, inhibitors of the transport molecule itself have been synthesized, some of which are currently undergoing preclinical and clinical testing. The main emphasis was thereby placed on specific inhibitors of the sugar reabsorption in the kidney. The following chapter briefly reviews the cellular and molecular basis of transepithelial sugar transport. It then summarizes the properties of the lead substance phlorizin and defines the pharmacologically important regions of the molecule. A description of the "phlorizin receptor" and its binding sites for phlorizin follows. Then screening procedures for sodium-D-glucose cotransporter (SGLT) inhibitors are introduced and the results of preclinical and clinical tests are compiled. In a synopsis, the current state of art in the development of SGLT inhibitors and their potential therapeutic use are discussed.

2 The Sodium-D-Glucose Cotransporter as Target

2.1 Role of Sodium-D-Glucose Cotransport in Transepithelial Sugar Transport

The SGLT plays a pivotal role in the translocation of sugars across epithelial membranes. In the small intestine and the renal tubule, transport of the sugar

is active and requires the coupling of cellular energy metabolism to the transepithelial translocation. The SGLT is the site where such coupling occurs. The coupling is not direct, i.e. there is no hydrolysis of ATP involved as in other so-called primary active transport events such as those mediated by ion translocating ATPases. Instead, the transporter uses the energy "stored" in an ion gradient to transport sugars against their concentration difference. Such processes are called secondary active and are used widely in unicellular and multicellular organisms. In mammalian species, the most prominent ion whose gradient across the cell membrane is used as driving force is sodium. The secondary active transport of organic substances – sugars, amino acids, carboxylic acids and inorganic ions such as chloride and phosphate involves the simultaneous movement of sodium ions – one, two or three – mostly in the same direction as the substrate in a symport mode. For vectorial transcellular transport also an asymmetry of the cell must be established, so that the plasma membrane facing one compartment has to contain different transporters than the membrane facing the other compartment into which translocation occurs (Kinne 1991).

Both of these elements are incorporated in current models on active transepithelial sugar transport. Only the apical membrane of the epithelial cell (termed brush border in the small intestine and in the renal proximal tubule) contains the SGLT. The sodium gradient across the brush border is generated by the sodium–potassium stimulated ATPase, a primary active ion transport ATPase which removes sodium from the cell interior in exchange to potassium ions. Thus, D-glucose can be accumulated in the epithelial cells uphill from the intestinal or renal tubular lumen above the sugar concentration in the blood. The sugar leaves the cell along its concentration difference in a carrier-mediated, sodium-independent, passive movement (Wright et al. 2007).

Sugar absorption in the human gut occurs in the first segments and there it has a high affinity and high velocity. As of the colon, sugar absorption ceases and sugar required for the intracellular metabolism enters the intestinal cells from the cell side exposed to the blood. Studies on the presence of SGLT1 mRNA and protein expression in the various intestinal segments confirmed and extended the knowledge on the intra-intestinal distribution. It should be noted that sugar transport in the intestine is also a mode to absorb sodium across the epithelium; therefore, the enteral application of a sodium–sugar solution is one of the most effective ways to compensate for the fluid and electrolyte loss in diarrhoea (Wright et al. 2007).

The other main organ where active sugar transport occurs is the kidney. In the early part of the proximal tubule, bulk reabsorption of filtered glucose against a small gradient occurs, in the late part residual glucose is removed against a steep concentration difference. Transport studies in the early and late segments of the proximal tubule as well as vesicle studies showed that the kidney contains two D-glucose cotransporters which differ in their stoichiometry for sodium in the early proximal tubule, one sodium and one sugar molecule are translocated together across the luminal membrane whereas in the late part two sodium ions are translocated with one sugar molecule. The two transporters also differ in their affinity for D-glucose; in the early part the apparent transport affinity (K_m) is about 2 mM, in the

late part the affinity is higher – K_m less than 0.5 mM. The two SGLTs also exhibit a different substrate specificity – distinction between D-galactose in the early part but not in the late part (Burckhardt and Kinne 1992).

The transporter in the late part is similar to the one found in the intestine termed SGLT1 whereas the one in the early proximal tubule is now referred to as SGLT2. The inherited disease of familial renal glucosuria can indeed be traced to a lack in SGLT2 in the early proximal tubule (Feld 2001; Calado et al. 2008). Interestingly, in these patients the reabsorptive capacity of the late proximal tubule seems to suffice to maintain a normal plasma glucose level. Thus, the only symptom is the increased urinary glucose excretion (Kleta et al. 2004). A lack of SGLT1 causes glucose–galactose malabsorption in the intestine with severe diarrhoea and salt and fluid loss (Wright et al. 2007).

2.2 *Molecular Basis of Sodium-D-Glucose Cotransport*

In 1987, Ernie Wright and his associates were able to identify the genetic message coding for the human intestinal SGLT by expression cloning using functional expression in oocytes and fractionation of the mRNA as a tool (Hediger et al. 1987). The gene codes for a protein of ~75 kDa which is heavily glycosylated. The glycosylation apparently does not affect its function but is probably related to its presence in the luminal brush border membrane, which is covered by an extensive glycocalyx.

Availability of the SGLT1 gene led thereafter to the identification of an identical gene in the kidney (Morrison et al. 1991) and to the similarity cloning of SGLT2 (Kanai et al. 1994). It also allowed performing mutagenesis studies to further elucidate the substrate binding sites, phosphorylation sites and inhibitor binding sites (Wright et al. 2007). In addition, overexpression in cells could be achieved which in the end led to the isolation of the transporter after heterologous expression in yeast (Tyagi et al. 2005).

The molecule responsible for the sodium-independent translocation of sugars has also been identified – there is a whole GLUT family whose properties have been reviewed recently. There is only a very limited sequence homology between SGLT and GLUT (Stuart and Trayhurn 2003).

Knowledge of the sequence also allowed for prediction and experimental analysis of the membrane topology of the transporter. The results of these studies consistently show that the N-terminus is pointing to the outside of the cell and that the molecule has 14 transmembrane segments. A major discrepancy exists concerning the topology at the C-terminus. Wright and associates position the loop between the 13th and 14th transmembrane segment (loop 13–14) into the cytoplasm (Hediger et al. 1987) whereas Puntheeranurak et al. have evidence for a location of at least the late part of the loop on the extracellular surface of the transporter (Puntheeranurak et al. 2006). The location of loop 13–14 is essential because, as

will be shown later, this loop appears to be part of the phlorizin binding site of the transporter.

The C-terminus also constitutes the transmembrane segments X–XIII of the transporter which are involved in sugar recognition and sugar translocation (Wright et al. 2007); the N-terminal segments IV–V are involved in sodium recognition as well as glucose binding and coupling of the sodium gradient to the sugar movement during cotransport.

2.3 Sugar Binding Sites of the SGLT

2.3.1 Substrate Specificity of the Sodium-D-Glucose Cotransporters

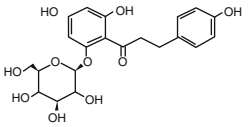
Extensive investigations on the essential features of the D-glucose molecule were undertaken to define the substrate specificity of transport. Only sugars in a hexose and the D-configuration are translocated whereas fructose as well as L-glucose and other sugars with the L-conformation are not transported. The positioning of the hydroxyl group at C1 is important as beta sugars are transported more avidly than alpha sugars. Short aliphatic and aromatic residues as in alpha-methyl D-glucose and arbutin (beta-phenyl D-glucoside) are also tolerated (Wright et al. 2007). Beta-glucosides with larger aromatic aglucones and the aglucones themselves are bound to the transporter and not translocated; they act as inhibitors of the transport.

The hydroxyl group at C2 has to be present in an equatorial position, thus 2-deoxy D-glucose, mannose and N-glucosamine are not transported. The presence of the hydroxyl group at C3 and its positioning has also some effect on transport, but 3-deoxy D-glucose, allose and 3-O-methyl D-glucose are transported although with a lower affinity and velocity. Galactose with a modification at the hydroxyl group at C4 is transported by SGLT1 but only to a limited extent by SGLT2. The hydroxyl group at C6 is of minor importance, 6-deoxy-glucose is transported by both SGLTs. At position C6, the SGLT2 seems to have an interesting additional hydrophobic binding site with a rather high binding capacity. Thus, C6 alkyl residues have a higher affinity for SGLT2 than for SGLT1 (Kipp et al. 1997).

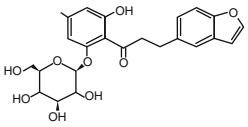
It has to be pointed out that the substrate specificity at the inner or cytoplasmic side of the carrier is different. At the cytoplasmic side, the selectivity with regard to L-glucose is reduced, the affinity for sugars is very much lower and the sodium sensitivity is also considerably decreased (Firnges et al. 2001).

There are differences in the substrate specificity of the overall transport process and the initial binding events at the outer surface of the transporter that precede the translocation. At the initial binding site, the primary sorting of the sugars according to the D- or L-configuration and the presence of the hydroxyl group at C3 occurs. At the entry to the translocation step, a further selection of the sugars with regard to the position and presence of the OH-groups at the other C-atoms takes place (Puntheeranurak et al. 2007b; Tyagi et al. 2011).

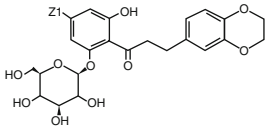
O-glucosides



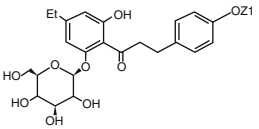
1



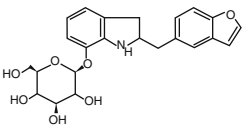
2



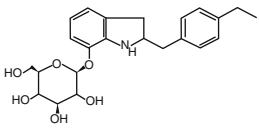
3



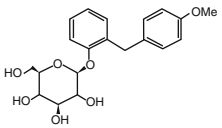
4



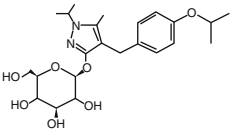
5



6

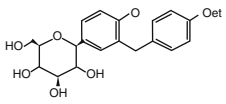


7

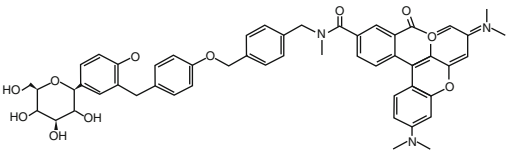


8

C-arylglucosides

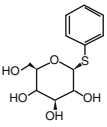


9

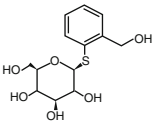


10

S-glycosides



11



12

Fig. 1 Chemical structure of phlorizin and some SGLT inhibitors including O-glucosides, C-arylglucosides and S-glycosides. For details see text

2.3.2 Sugar Binding Site(s) of the Sodium-D-Glucose Cotransporter

According to mutagenesis studies, amino acids involved in sugar transport are clustered in transmembrane segments X, XI, XII and XIII. These amino acids are accessible from the outside of the membrane and are located in a hydrophilic pocket (Tyagi et al. 2007). The binding pocket has a depth of about 7 Å. A recent publication on the structure of an archetype of a sugar sodium cotransporter confirms the assumption of the presence of a hydrophilic pocket in the outside-facing conformation and in the inside-facing conformation. The gates between these two pockets are formed by clusters of hydrophobic amino acids (Faham et al. 2008).

Atomic force microscopy (AFM) studies combined with accessibility and mutagenesis studies have recently shown that disulfide bridges between extramembranous loops are also important in forming the initial sugar binding site at the carrier. Thus, extramembranous loops 6–7 and 13–14 seem to be interconnected by a disulfide bridge to form a binding pocket which also brings loop 8–9 and TMs VI to XIII closely together, thereby facilitating the formation of the sugar translocation pathway (Puntheeranurak et al. 2007a; Tyagi et al. 2011).

3 The Prototype of SGLT Inhibitors: Phlorizin

3.1 General Remarks

The structure of the most frequently studied O-glucoside inhibitor of the SGLT, phlorizin, is shown in Fig. 1 (compound **1**). Phlorizin, a beta glucoside derived from the bark of apple roots inhibits glucose transport in the intestine and in the kidney (Ehrenkranz et al. 2005). The affinity of phlorizin in transport studies, i.e. in the presence of D-glucose is about 1 μM for hSGLT1 and 20 nM for hSGLT2 (Pajor et al. 2008). Phlorizin was also shown to competitively inhibit the transport without evidence that it is translocated across the membrane. In a series of transport studies in microperfused rat renal tubules (Vick et al. 1973), it was found that the affinity of phlorizin congeners mirrored the stereospecific requirements for sugar translocation, supporting the view that interaction of phlorizin with the transporter occurs at the sugar binding/translocation site of the transporter. On the other hand, the nature of the aglucone influenced the inhibitory potency. It had also been observed previously that phloretin, the aglucone of phlorizin inhibits sugar transport but in a non-competitive way and with a low affinity (Vick et al. 1973). Thus, it was concluded that phlorizin binds to the outside of the SGLT at two domains, one represents the sugar binding site and the other acts as an aglucone binding site. These multiple interactions can explain the high affinity of phlorizin compared to the substrate D-glucose.

3.2 SGLT as Phlorizin Receptor

3.2.1 Binding Studies on Brush Border Membranes

The availability of radioactively labelled phlorizin made it possible to study the interaction between phlorizin and the SGLT directly. On isolated brush border membranes of rat kidney high affinity binding of phlorizin could be demonstrated, which was sodium dependent and competitively inhibited by D-glucose but not by L-glucose. The maximum amount of phlorizin bound specifically to the brush border membrane was found to be 10 nmol/mg membrane protein and the number of receptors (transporters) per apical cell surface was estimated to be about 6,000 per cell (Bode et al. 1970). Sodium-dependent, D-glucose-inhibitable binding of phlorizin was also used in our laboratory as a signal in initial attempts to purify the transporter, as was the interaction with a phlorizin polymer.

It should be noted here that for the binding studies renal but not intestinal brush border membranes had to be used, because the renal membranes do not contain the disaccharidases that hydrolyze O-glycosides in the intestine.

3.2.2 Interactions of Phlorizin with the Isolated Transporter and Its Subdomains

Heterologous expression of hSGLT1 in yeast and isolation of the protein by affinity chromatography made it possible to study the interactions of the phlorizin receptor with phlorizin in more detail. As determined by tryptophan fluorescence experiments, phlorizin is bound to the isolated receptor in a sodium-dependent manner with an affinity of about 5 μM. It also could be shown that phlorizin but not phloretin induces the same conformational changes in the substrate binding site as D-glucose, adding proof to the assumption of an interaction of the glucose moiety of phlorizin with the sugar binding site of the phlorizin receptor (Tyagi et al. 2007).

The location of the aglucone binding site was investigated initially in rabbit SGLT1 by mutagenesis and transport studies in transfected cells which showed that the region between aa 602 and 610 is critically involved in phlorizin binding but not in sugar binding (Novakova et al. 2001). The same conclusion was reached in Trp fluorescence studies on the isolated transporter (hSGLT1) which showed that positions 602 and 609 undergo major changes in conformation when phlorizin or phloretin is present in the solution but undergo only minor changes in the presence of D-glucose (Tyagi et al. 2007). Evidence for a location on the surface of the transporter was derived from AFM studies using cantilever tips primed with an antibody against aa 606 to 630. The antibody interacted specifically with rbSGLT1 in intact cells under physiological conditions suggesting that this region of the receptor is accessible from the extracellular site. In the presence of phlorizin, the probability of binding of the antibody was drastically reduced indicating a strong conformational change in this region of the transporter. The aa 606 to 630

represent the late part of loop 13–14 – presumably one of the extramembranous loops of the transporter – as discussed earlier (Puntheeranurak et al. 2007a).

The molecular mechanism of the binding of phlorizin was further investigated on isolated subdomains of rbSGLT1. Binding studies with loop 13 in solution confirmed a specific interaction of phlorizin with this domain of the transporter. Phlorizin unfolds part of the loop and then brings the two small helical segments closely together, leading to a condensed conformation of the loop (Raja et al. 2003). Such condensation – increased hydrophobicity of the peptides – is also observed in the complete isolated carrier (hSGLT1) in the presence of phlorizin. Alkylglucosides which have also been found to inhibit SGLT bind with a high affinity to the isolated loop, they induce a different but also condense conformation (Kipp et al. 1997).

3.2.3 Differences Between hSGLT1 and hSGLT2

For the discussion in this chapter, it is of interest to compare the sequence of SGLT1 and SGLT2. As expected there are many sequence similarities or conservative replacements and identities in particular in the transmembrane segments. The extramembranous loops are more variable, in particular in the C-terminus where the aglucone binding site of the phlorizin receptor is located (Althoff et al. 2006). Thus, loop 13–14 of the hSGLT2 has ten more amino acids than hSGLT1 and two more cysteines close to the assumed binding site. In addition, several hydrophilic amino acids have been replaced by hydrophobic residues. Only recently, the two human transporters were compared with regard to the properties and the importance of the amino acids in the aglucone binding region. Pajor et al. (2008) described that hSGLT2 has a higher affinity for the aglucone phloretin than hSGLT1, confirming differences in the properties of the binding site and explaining the higher affinity of hSGLT2 for phlorizin. Furthermore, mutation of the conserved cysteine in position 610 in hSGLT1 led, as expected, to a decrease in the affinity of the transporter to phlorizin whereas mutation of the equivalent Cys 615 in hSGLT2 caused an increase in affinity. Thus, the two transporters differ significantly not only in their sugar binding sites but also in the conformation and the physical chemical properties of their aglucone binding sites.

3.3 Pharmacophore Analysis and Dimensions of the Phlorizin Binding Pocket

In a study combining 2D-NMR, molecular dynamics and pharmacophore analysis, the essential elements for the interaction of phlorizin with its binding pocket in nonhuman SGLTs were determined. The most probable phlorizin conformation shows a nearly perpendicular arrangement of the two aromatic rings (A and B) with the ring B situated above the sugar ring. As shown in Fig. 2, hydrogen bonding via

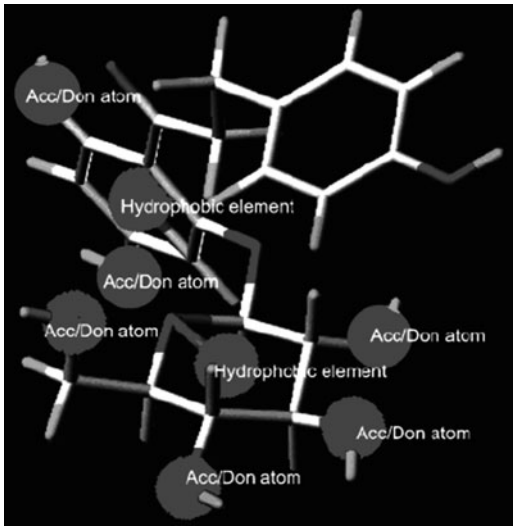


Fig. 2 Pharmacophore analysis of phlorizin (*white bars* carbon atoms, *grey bars* hydrogen atoms, *black bars* oxygen atoms and *circles* pharmacophoric elements) (reprinted from Wielert-Badt et al. 2000)

hydroxyl groups of the glucose moiety at C(2), C(3), C(4) and C(6) and at C(4) and C(6) of aromatic ring A and hydrophobic interactions via the pyranoside ring and aromatic ring A are the essential features. From these conformational features of the pharmacophore, the dimension of the phlorizin binding site on the SGLT was estimated to be 13 × 10 × 7 Å (Wielert-Badt et al. 2000). Combined with the fluorescence studies mentioned earlier, the 7 Å probably correspond to the hydrophilic pocket into which the sugar molecules and the sugar moiety of phlorizin orient themselves. The area above the pocket probably represents the (mostly hydrophobic) aglucone binding site on the surface of the transporter (late part of loop 13–14). A tentative model of the phlorizin binding site of hSGLT1 is shown in Fig. 3. The sugar moiety thereby interacts with amino acids in the sugar binding pocket of the transporter whereas the aglucone moiety interacts with amino acids located on the surface of the transporter in the late part of loop 13–14 (Tyagi et al. 2007, 2011). In rat kidney also the hydroxyl group at the 6 position of the B ring proved to be important for the action of various compounds on the glucose reabsorption (Hongu et al. 1998b).

4 Synthesis and Screening of Derivates of Phlorizin

4.1 O-glucosides, C-arylglucosides, N-glucosides and S-glycosides

Phlorizin (glucose, 1-[2-(β-D-glucopyranosyloxy)-4,6-dihydroxyphenyl]-3-(4-hydroxyphe-nyl)-1-propanone, compound **1** in Fig. 1) can be considered as the

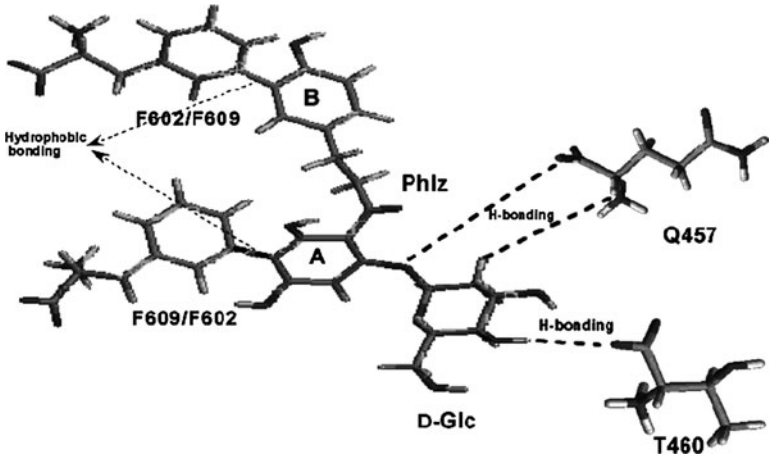


Fig. 3 Hypothetical scheme of major interaction sites between phlorizin (Phlz) and hSGLT1. The sugar moiety of phlorizin interacts with residues Gln457 and Thr460 present in transmembrane helix XI probably by the same hydrogen bond interactions as D-glucose does; the aromatic ring A of the aglucone interacts with Phe609/Phe602, and ring B makes contact with Phe602/Phe609; both are present in the extracellular loop 13 of hSGLT1 (reprinted from Tyagi et al. 2005)

lead substance for inhibitors of SGLT. Despite the observed anti-diabetic effect of phlorizin, it is not suited as a potential drug for the treatment of diabetes because of its low absorption caused by the hydrolysis of the O-glycosidic bond by intestinal disaccharidases. As a consequence, other glycosides have been developed some of which are shown in Fig. 1. One of these substances is T-1095 (compound **2**: 3-(benzo[*b*]furan-5-yl)-2,6-dihydroxy-4-methyl propiophenone 2-*O*-(6-*O* methoxy-carbonyl-β-D-glucopyranoside), a phlorizin-based SGLT inhibitor (Adachi et al. 2000; Arakawa et al. 2001; Oku et al. 1999, 2000a; Tsujihara et al. 1999). T-1095 has been shown to correct elevated blood glucose levels in rodents (Oku et al. 1999, 2000c; Ueta et al. 2005, 2006).

In glucosylated dihydrochalcones modification of the benzofuran moiety to benzodioxane (compound **3**) or 4-ethoxyphenyl (compound **4**), the 4-substituent of the phenyl ring improve SGLT2 selectivity. Another example is the modification of the ketone/phenol moiety of compound 2 leading to the indole-O-glucosides (compound **5** and compound **6**). Recently, canagliflozin, a novel C-glucoside with a thiophene ring has been added as potential drug. Also N-glucosides have been considered as interesting compounds (Washburn 2009).

Finally, thioglycosides, such as phenyl-1-thio-β-D-glucopyranoside (compound **11**) and 2-hydroxymethyl-phenyl-1-thio-β-D-galacto-pyranoside (compound **12**), have been shown to exert a pronounced inhibitory effect on SGLT2 and SGLT1, respectively (Castaneda et al. 2007)

Several SGLT inhibitors are currently in clinical trials. This is the case for sergliflozin (compound **7**) and dapagliflozin. Sergliflozin is a benzylphenol glucoside (Katsuno et al. 2007) and dapagliflozin is a C-aryl glucoside (Meng et al. 2008).

Dapagliflozin is the SGLT2 inhibitor with the most clinical data available to date, with other SGLT2 inhibitors currently in the developmental pipeline. It has demonstrated sustained, dose-dependent glucosuria over 24 h with once-daily dosing in clinical trials (Hanefeld and Forst 2010; Neumiller et al. 2010).

4.2 Screening Methods

4.2.1 Cellular Assays

The activity of SGLT has been analyzed by different methods, including transport assays with radioactively labelled sugars in brush border membrane vesicles (Kinne et al. 1975; Murer et al. 1974), *Xenopus* oocytes (Parent et al. 1992a) and transiently or permanently transfected cells (Brot-Laroche et al. 1987). The *Xenopus* oocyte expression system in combination with voltage-clamp techniques has yielded important details on the mechanisms underlying the binding and translocation reactions of the transporter (Kimmich 1990; Parent et al. 1992b; Sakhrani et al. 1984; Wright et al. 2007). One of the most widely used cell line is the Chinese Hamster Ovary (CHO) cell line (Lin et al. 1998; Sakhrani et al. 1984). Stably transfected CHO cells expressing hSGLT1 or hSGLT2 (Castaneda and Kinne 2005) or transiently transfected CHO (Katsuno et al. 2007) and COS-7 cells (Pajor et al. 2008) have also been used for functional characterization and screening of mutants and analysis of inhibitors.

Current methods that use radioactive labelled substances, however, are expensive due to the amount of radioactive compounds required and the large amount of substance necessary for inhibitor-type studies. For these reasons, we developed an alternative 96-well automated method to study the activity of hSGLT1 and hSGLT2 using stably transfected CHO cells (Castaneda and Kinne 2005). The advantage of using the 96-well method is the low amount of radioactive compounds and inhibitory substances required, as well as the ability to establish reproducibility because of the repetition built into the assay. Furthermore, this method can easily be performed semi-automatically to yield quantitative data regarding key aspects of glucose membrane transport and kinetic studies of potential inhibitors of human SGLT1 and SGLT2.

In addition to electrophysiology and radioactivity, fluorescence methods have also been reported. Fluorescence resonance energy transfer has been used to monitor changes of membrane potential as an indicator of uptake caused by SGLT (Castaneda et al. 2007; Weinglass et al. 2008). Also, fluorophore-conjugated SGLT2 inhibitors have been synthesized (compound **10**, Fig. 1) (Landsdell et al. 2008). It has been suggested that these compounds could be used in binding studies. Fluorescence presents some advantages over radioactivity, specifically with regard to waste disposal and safety. Furthermore, fluorescence assays can be used for high-throughput screening (Weinglass et al. 2008).

4.2.2 Animal Models

Animals have been used widely as experimental models in diabetes research. The importance of animal models in this type of research was demonstrated in studies using pancreatectomised dogs. These studies revealed the essential role of the pancreas and insulin in glucose homeostasis (Finkelstein et al. 1975). They also provide important information about pharmacokinetics, pharmacodynamics, ADME and toxicity. All of this information is mandatory to continue with clinical trials, which is the next and last step in drug development.

Each of these models is characterized by the presence of specific metabolic alterations found in diabetes such as hyperglycaemia, obesity, early hyperinsulinaemia and insulin resistance (Berglund et al. 1978; Herberg and Coleman 1977). One of the most used animal models is the Zucker diabetic fatty (ZDF) rat model (Zhang et al. 2006). However, other specific models for the study of glucose intolerance and diabetic complications have also been developed. For example, Goto-Kakizaki (GK) rats have been developed by selectively breeding them from non-diabetic Wistar rat with glucose intolerance (Goto et al. 1988). In addition, the C57BL/KsJ-db/db mouse represents a diabetic nephropathy animal model that is often used (Arakawa et al. 2001).

Several studies using animal models reported the effect of SGLT inhibitors for the treatment of diabetes. For example, chronic subcutaneous administration of phlorizin has been shown to reduce plasma glucose levels in diabetic rodents (Jonas et al. 1999). T-1095 reduces blood glucose levels in both type 1 (Adachi et al. 2000; Oku et al. 1999) and type 2 (Arakawa et al. 2001; Oku et al. 1999; Ueta et al. 2005) diabetic animal models. Canagliflozin also showed pronounced anti-hyperglycaemic effects in high-fat fed KK-mice (Nomura et al. 2010).

5 Therapeutic Efficacy of SGLT Inhibitors

5.1 In Vitro Studies on Sugar Transport by Cultured Transfected Cells

The first evidence for the potential of glucosides in inhibiting glucose transport is obtained in in vitro cell-based studies (Ohsumi et al. 2003; Oku et al. 1999). These studies represent the first stage in drug discovery and development. The concentration required for 50% inhibition of the [^{14}C]AMG-uptake rate (IC_{50}) represents an important indicator of the potential therapeutic effect of SGLT inhibitors for the control of hyperglycaemia. The SGLT inhibitors exert their action by competitive inhibition. For this reason, the inhibitory constant (K_i) can also be used as an indicator of the effect of SGLT inhibitors. The IC_{50} values and K_i values of SGLT inhibitors described in the literature are given in Table 1. In addition to their affinity to the transporter – which determines the dose of the compound – the

Table 1 Effect of O-glucosides, C-arylglucosides and S-glucosides based on IC50 values obtained in in vitro studies. For structure of compounds see Fig. 1

Compound	hSGLT1 (IC_{50}), μM	hSGLT2 (IC_{50}), μM	SGLT1/ SGLT2	Therapeutic effect	References
O-glucosides					
1	0.16	0.16	1	–	Oku et al. 1999
2	0.20	0.05	4	+	Oku et al. 1999[b]
3	6.28	0.015	418.7	n.d.	Dudash et al. 2004[a]
4	8.39	0.034	246.8	n.d.	Dudash et al. 2004[a]
5	0.145	0.024	6	n.d.	Zhang et al. 2006
6	2.14	0.028	76.4	n.d.	Zhang et al. 2006
7	2.1	0.010	210	+	Pajor et al. 2008[b]
8	4.5	0.012	364.5	+	Fujimori et al. 2008
C-arylglucosides					
9	1.4	0.001	1,263.6	+	Meng et al. 2008[b]
10	n.d.	0.055	n.d.	n.d.	Landsdell et al. (2008)
S-glucosides					
11	30	10	3	n.d.	Castaneda et al. 2007
12	14	88	0.2	n.d.	Castaneda et al. 2007

[a] K_i

[b] Used in clinical trials

Recently, also the results of transport studies with canagliflozin have been published (Nomura et al. 2010). The IC_{50} for hSGLT2 is 2.2 nmol and the specificity ratio is 413

selectivity for SGLT2 is important, since inhibition of glucose reabsorption in the kidney but not of the sugar absorption in the intestine has to be achieved. Therefore, the ratio of the affinities for SGLT1 and SGLT2 is also given in the table. According to both criteria compound **9** seems to be optimal, it shows a high affinity to SGLT2 combined with high selectivity for SGLT2. For these reasons, compound **9** (Fig. 1) seems to be a promising therapeutic substance for the treatment of hyperglycaemia.

5.2 Effect of SGLT Inhibitors in Preclinical and Clinical Studies

5.2.1 Urinary Glucose Excretion

The SGLT inhibitors induce a dose-dependent increase of urinary glucose excretion in different diabetic animal models including rats, mice and dogs (Adachi et al. 2000; Arakawa et al. 2001; Ellsworth et al. 2008; Oku et al. 1999, 2000b; Ueta et al. 2005). Inhibition of SGLT2 activity reduces tubular glucose reabsorption and due to the reduction of TmG, excess plasma glucose is excreted into the urine (see Fig. 4), thus reducing hyperglycaemia after glucose loading (Katsuno et al. 2007; Tsujihara et al. 1996, 1999). Due to the selective inhibition of SGLT2 a residual reabsorption of about 20–25% of the filtered load for glucose remains, attributable to the operation of SGLT1 in the late proximal tubule. The effect of SGLT inhibitors on urinary glucose excretion has also been associated with reduction of

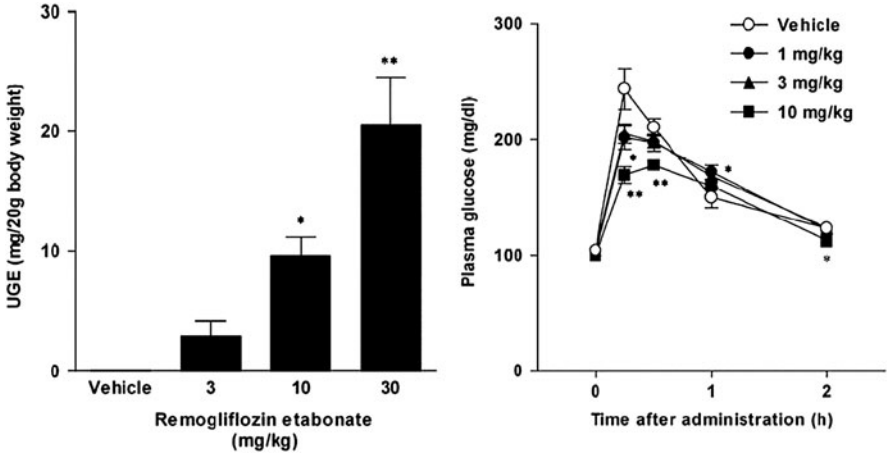


Fig. 4 Effect of SGLT2 inhibitor remogliflozin on urinary glucose excretion (UGE) and on plasma glucose during an oral glucose tolerance test (reprinted from Fujimori et al. 2008). The obtained UGE for vehicle was zero

body weight gain in diabetic rats resulting in a sustained improvement of hyperglycaemia (Ueta et al. 2005).

5.2.2 Effect on Plasma Glucose Levels

The SGLT inhibitors regulate blood glucose levels in a dose-dependent manner (Adachi et al. 2000; Katsuno et al. 2007). This effect is similar to that observed with other anti-diabetic drugs, such as insulin secretagogues or glucosidases, and results in improvements of postprandial hyperglycaemia (Ichikawa et al. 2002; Ikenoue et al. 1997). In addition to reducing blood glucose, SGLT inhibitors also decrease HbA_{1c} levels. This effect improves glucose intolerance and insulin resistance, and prevents the development of diabetic neuropathy in Goto-Kakizaki rats (Ueta et al. 2005).

5.2.3 Effect on Fasting Hypoglycaemia

One of the most severe potential side effects of anti-diabetic drugs is the induction of fasting hypoglycaemia. Animal studies demonstrated that SGLT inhibitors, such as T-1095 (Adachi et al. 2000; Oku et al. 2000c) and sergliflozin (Katsuno et al. 2007), do not cause hypoglycaemia (equivalent to plasma glucose level below 70 mg/dl) even in a 6-h fasting condition. The reason for this behaviour is that the inhibitors used mainly inhibit SGLT2 and not SGLT1. As evident from the familial renal glucosuria, even a complete absence of SGLT2 does not lead to hypoglycaemia (Kleta et al. 2004) because the residual reabsorption of glucose in

the late proximal tubule mediated by SGL1 suffices to maintain close to normal plasma glucose level. Therefore, the risk of hypoglycaemia with these drugs is predicted to be low, making them potentially safe drugs even in the instance of an overdose.

6 Benefits and Pitfalls of SGLT Inhibitors

6.1 Benefits

The SGLT2 represents an important molecular target for the treatment of diabetes because it plays a major role in renal glucose reabsorption and its tissue distribution is limited to the kidney, thus reducing side effects.

It has been suggested that the effect of SGLT inhibitors on blood glucose control via an increase in urinary glucose excretion results in negative energy balance with body weight control and preservation of insulin secretion (Katsuno et al. 2007). Weight reduction and improved insulin sensitivity caused by SGLT inhibitors would offer some advantages over other anti-diabetic drugs, such as sulfonylureas, α-glucosidase inhibitors, thiazolidinediones and biguanides. These effects would be particularly important for the treatment of type 2 diabetes.

The SGLT inhibitors correct both hyperglycaemia and energy imbalance (Hongu et al. 1998a, b; Tsujihara et al. 1996). A negative energy balance contributes to a reduction of diabetes complications without necessarily decreasing body weight or the risk of hypoglycaemia (DeFronzo 2004). In addition to the effect on hyperglycaemia with reduction of diabetic complications observed with the use of SGLT inhibitors, these inhibitors have also been found to be associated with restoration of impaired insulin secretion from pancreatic ß-cells (DeFronzo 2004).

Another important characteristic of SGLT inhibitors is that they do not alter the glucose transport via GLUT. As a consequence, glucose delivery to the brain, liver and muscle is not affected. Moreover, the high specificity of some SGLT inhibitors, such as sergliflozin (Katsuno et al. 2007), dapagliflozin (Meng et al. 2008) and canagliflozin (Nomura et al. 2010), precludes any negative gastrointestinal side effects associated with SGLT1 inhibition.

6.2 Pitfalls

The expression of SGLT1 in other tissues in addition to the kidney represents a restriction for the use of SGLT inhibitors that are not selective to SGLT2. The SGLT1 is expressed in the capillary endothelial cells of rat heart and muscle (Wright et al. 2007), in capillary endothelial cells of the blood brain barrier in rat (Elfeber et al. 2004) as well as in other tissues (trachea, testis, prostate, mammary

and salivary glands) (Kinne unpublished; Wright et al. 2007). Therefore, the effect of SGLT inhibitors on other tissues in which SGLT is expressed must be further investigated.

Another important factor that remains to be evaluated is the effect of glycosuria and osmotic diuresis induced by SGLT inhibitors. Glucosuria has been reported as a risk factor associated with bacterial urinary tract infection. Interestingly, the concentration of glucose excreted into the urine is higher if bacterial growth has been observed (Tsujihara et al. 1999). However, patients with familial renal glucosuria due to a defect in the SGLT2 gene remain asymptomatic (van den Heuvel et al. 2002; Scholl-Burgi et al. 2004). Furthermore, altered bladder function secondary to diabetes seems to play an additional role in the development of urinary tract infections (Patterson and Andriole 1995) and not only the amount of glucose excreted into the urine. For that reason, clinical trials are necessary to evaluate the role of SGLT inhibitors in the development of urinary tract infections.

The SGLT inhibitors, such as sergliflozin, produce a slight and temporary diuretic effect when they are used at high concentrations (equivalent to 30 mg/kg) (Katsuno et al. 2007). At low concentration (of about 3 or 10 mg/kg), however, no significant change in urinary volume has been reported. Osmotic diuresis in normoglycaemic conditions remains asymptomatic but under hyperglycaemic conditions might be associated with adverse reactions (Venkatraman and Singhi 2006). Osmotic diuresis leads to depletion of intravascular volume and decreased renal perfusion. As a result, the amount of glucose that can be excreted is reduced (Adrogue 1992). The resultant hyperosmolarity and dehydration stimulates the production of cortisol, catecholamines and glucagon, and further increases the degree of hyperglycaemia (Kitabchi et al. 2001). These side effects need to be investigated further to better understand the safety of SGLT inhibitors.

7 Current State and Future Developments

Based on the observed effects of different SGLT inhibitors in diabetes and hyperglycaemia, they have been proposed as potential therapeutic substances and new compounds are currently under investigation. One of these compounds was a benzylpyrazole glucoside known as remogliflozin (compound **8**, Fig. 1) (Fujimori et al. 2008). Studies performed in vitro and using diabetic animal models have demonstrated that remogliflozin is a potent and highly selective SGLT2 inhibitor, as shown by increased urinary glucose excretion, reduced fasting plasma glucose and glycated haemoglobin levels, improved hyperglycaemia, hyperinsulinaemia, hypertriglyceridaemia and insulin resistance in rodents (Fujimori et al. 2008). However, its further development has been discontinued by GlaxoSmithKline (Kissei Pharmaceutical Co. Ltd 2009). The most promising candidate is currently dapagliflozin (Hanefeld and Forst 2010).

Another substance that currently is under investigation is desoxyrhaponticin, an O-glucoside, found in extracts of the rhubarb plant. Studies in diabetic rats

demonstrated that desoxyrhaponticin has an inhibitory effect on SGLT (Li et al. 2007). The exact mechanism of action of this substance remains to be determined.

The SGLT2 represents a promising molecular target for the development of new alternatives in the treatment of diabetes based on the following aspects: (a) SGLT2 is expressed exclusively in the renal proximal tubules, and thus a selective SGLT2 inhibitor should not affect other tissues and (b) enhancement of urinary glucose excretion via SGLT2 inhibition leads to a negative energy balance, which currently is not really achieved by any existing clinical pharmacological intervention. Inhibition of glucose transport in the kidney through SGLT inhibitors represents a different mechanism of action from other hypoglycaemic agents. For these reasons, SGLT inhibitors should be considered as a potential new therapeutic alternative for the treatment of diabetes either alone or in combination with other anti-diabetic drugs. Moreover, therapeutic alternatives to achieve a reduction of glucose reabsorption in the proximal tubules, which represents the last step in the fate of glucose in the organism, represent an additional strategy for the treatment of diabetes that needs to be investigated further.

Acknowledgments The authors would like to thank Christine Riemer, for her outstanding organizational skill and computer proficiency, as well as her endurance and patience. Without her, the writing of this chapter while travelling between two continents would not have been possible. Also, the enthusiastic and untiring support of the staff of the library of the Max Planck Institute, particularly Christiane Berse and Jürgen Block is gratefully acknowledged. In the analysis of the differences of the aglucone binding site of hSGLT1 and hSGLT2, Dr. Thorsten Althoff, now at the Max Planck Institute of Biophysics in Frankfurt, Germany, provided essential help.

References

Adachi T, Yasuda K, Okamoto Y, Shihara N, Oku A, Ueta K, Kitamura K, Saito A, Iwakura I, Yamada Y, Yano H, Seino Y, Tsuda K (2000) T-1095, a renal Na+-glucose transporter inhibitor, improves hyperglycemia in streptozotocin-induced diabetic rats. Metabolism 49: 990–995

Adrogue HJ (1992) Glucose homeostasis and the kidney. Kidney Int 42:1266–1282

Althoff T, Hentschel H, Luig J, Schutz H, Kasch M, Kinne RK (2006) Na+-D-glucose cotransporter in the kidney of Squalus acanthias: molecular identification and intrarenal distribution. Am J Physiol Regul Integr Comp Physiol 290:R1094–R1104

Arakawa K, Ishihara T, Oku A, Nawano M, Ueta K, Kitamura K, Matsumoto M, Saito A (2001) Improved diabetic syndrome in C57BL/KsJ-db/db mice by oral administration of the Na^+-glucose cotransporter inhibitor T-1095. Br J Pharmacol 132:578–586

Asano N (2003) Glycosidase inhibitors: update and perspectives on practical use. Glycobiology 13:93R–104R

Berglund O, Frankel BJ, Hellman B (1978) Development of the insulin secretory defect in genetically diabetic (db/db) mouse. Acta Endocrinol (Copenh) 87:543–551

Bode F, Baumann K, Frasch W, Kinne R (1970) Binding of phlorhizin to the brushborder fraction of rat kidney. Pflugers Arch 315:53–65

Brot-Laroche E, Supplisson S, Delhomme B, Alcalde AI, Alvarado F (1987) Characterization of the D-glucose/Na+ cotransport system in the intestinal brush-border membrane by using the specific substrate, methyl alpha-D-glucopyranoside. Biochim Biophys Acta 904:71–80

Burckhardt G, Kinne RKH (1992) Transport proteins. Cotransporters and countertransporters. In: Seldin DW, Giebisch G (eds) The kidney: physiology and pathophysiology. New York, Raven, pp 537–586

Calado J, Sznajer Y, Metzger D, Rita A, Hogan MC, Kattamis A, Scharf M, Tasic V, Greil J, Brinkert F, Kemper MJ, Santer R (2008) Twenty-one additional cases of familial renal glucosuria: absence of genetic heterogeneity, high prevalence of private mutations and further evidence of volume depletion. Nephrol Dial Transplant 12:1–6

Castaneda F, Kinne RKH (2005) A 96-well automated method to study inhibitors of human sodium-dependent D-glucose transport. Mol Cell Biochem 280:91–98

Castaneda F, Burse A, Boland W, Kinne RK (2007) Thioglycosides as inhibitors of hSGLT1 and hSGLT2: potential therapeutic agents for the control of hyperglycemia in diabetes. Int J Med Sci 4:131–139

Ceriello A (2005) PROactive Study: (r)evolution in the therapy of diabetes? Diabet Med 22: 1463–1464

de Melo EB, Gomes AD, Carvalho I (2006) alpha- and beta-Glucosidase inhibitors: chemical structure and biological activity. Tetrahedron 62:10277–10302

DeFronzo RA (2004) Pathogenesis of type 2 diabetes mellitus. Med Clin North Am 88:787

Dudash J Jr, Zhang X, Zeck RE, Johnson SG, Cox GG, Conway BR, Rybczynski PJ, Demarest KT (2004) Glycosylated dihydrochalcones as potent and selective sodium glucose co-transporter 2 (SGLT2) inhibitors. Bioorg Med Chem Lett 14(20):5121–5125

Ehrenkranz JRL, Lewis NG, Kahn CR, Roth J (2005) Phlorizin: a review. Diab Metab Res Rev 21:31–38

Elfeber K, Stumpel F, Gorboulev V, Mattig S, Deussen A, Kaissling B, Koepsell H (2004) Na+-D-glucose cotransporter in muscle capillaries increases glucose permeability. Biochem Biophys Res Commun 314:301–305

Ellsworth BA, Meng W, Patel M, Girotra RN, Wu G, Sher PM, Hagan DL, Obermeier MT, Humphreys WG, Robertson JG, Wang A, Han S, Waldron TL, Morgan NN, Whaley JM, Washburn WN (2008) Aglycone exploration of C-arylglucoside inhibitors of renal sodium-dependent glucose transporter SGLT2. Bioorg Med Chem Lett 18:4770–4773

Faham S, Watanabe A, Besserer GM, Cascio D, Specht A, Hirayama BA, Wright EM, Abramson J (2008) The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na^+/sugar symport. Science 3:1–5

Feld LG (2001) Renal glycosuria. e Med J 2:1–6

Finkelstein SM, Bleicher MA, Batthany S, Tiefenbrun J (1975) In vivo modeling for glucose homeostasis. IEEE Trans Biomed Eng 22:47–52

Firnges MA, Lin JT, Kinne RKH (2001) Functional asymmetry of the sodium-D-glucose cotransporter expressed in yeast secretory vesicles. J Membr Biol 179:143–153

Fujimori Y, Katsuno K, Nakashima I, Ishikawa-Takemura Y, Fujikura H, Isaji M (2008) Remogliflozin etabonate, in a novel category of selective low-affinity / high-capacity sodium glucose cotransporter (SGLT2) inhibitors, exhibits antidiabetic efficacy in rodent models. J Pharmacol Exp Ther 327:268–276

Goto Y, Suzuki K, Ono T, Sasaki M, Toyota T (1988) Development of diabetes in the non-obese NIDDM rat (GK rat). Adv Exp Med Biol 246:29–31

Hanefeld M, Forst T (2010) Dapagliflozin, an SGLT2 inhibitor, for diabetes. Lancet 375: 2196–2198

Hediger MA, Coady MJ, Ikeda TS, Wright EM (1987) Expression cloning and cDNA sequencing of the Na^+/glucose co-transporter. Nature 330:379–381

Herberg L, Coleman DL (1977) Laboratory animals exhibiting obesity and diabetes syndromes. Metabolism 26:59–99

Hongu M, Funami N, Takahashi Y, Saito K, Arakawa K, Matsumoto M, Yamakita H, Tsujihara K (1998a) Na+-glucose cotransporter inhibitors as antidiabetic agents. III. Synthesis and pharmacological properties of 4'-dehydroxyphlorizin derivatives modified at the OH groups of the glucose moiety. Chem Pharm Bull 46:1545–1555

Hongu M, Tanaka T, Funami N, Saito K, Arakawa K, Matsumoto M, Tsujihara K (1998b) Na+-glucose cotransporter inhibitors as antidiabetic agents. II. Synthesis and structure-activity relationships of 4'-dehydroxyphlorizin derivatives. Chem Pharm Bull 46:22–33

Ichikawa K, Yamato T, Ojima K, Tsuji A, Ishikawa K, Kusama H, Kojima M (2002) Effect of KAD-1229, a novel hypoglycaemic agent, on plasma glucose levels after meal load in type 2 diabetic rats. Clin Exp Pharmacol Physiol 29:423–427

Ikenoue T, Okazaki K, Fujitani S, Tsuchiya Y, Akiyoshi M, Maki T, Kondo N (1997) Effect of a new hypoglycemic agent, A-4166 [(-)-N-(trans-4-isopropyl-cyclohexanecarbonyl)-D-phenylalanine], on postprandial blood glucose excursion: Comparison with voglibose and glibenclamide. 7. Biol Pharm Bull 20:354–359

Jonas JC, Sharma A, Hasenkamp W, Ilkova H, Patane G, Laybutt R, Bonner-Weir S, Weir GC (1999) Chronic hyperglycemia triggers loss of pancreatic beta cell differentiation in an animal model of diabetes. J Biol Chem 274:14112–14121

Kanai Y, Lee WS, You G, Brown D, Hediger MA (1994) The human kidney low affinity Na+/glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose. J Clin Invest 93:397–404

Katsuno K, Fujimori Y, Takemura Y, Hiratochi M, Itoh F, Komatsu Y, Fujikura H, Isaji M (2007) Sergliflozin, a novel selective inhibitor of low-affinity sodium glucose cotransporter (SGLT2), validates the critical role of SGLT2 in renal glucose reabsorption and modulates plasma glucose level. J Pharmacol Exp Ther 320:323–330

Kimmich GA (1990) Membrane potentials and the mechanism of intestinal Na(+)-dependent sugar transport. J Membr Biol 114(1):1–27. Review

Kinne RKH (1991) Selectivity and direction – plasma-membranes in renal transport. Am J Physiol 260:F153–F162

Kinne R, Murer H, Kinne-Saffran E, Thees M, Sachs G (1975) Sugar-transport by renal plasma-membrane vesicles – Characterization of systems in brush-border microvilli and basal-lateral plasma-membranes. J Membr Biol 21:375–395

Kipp H, Kinne-Saffran E, Bevan C, Kinne RK (1997) Characteristics of renal Na+-D-glucose cotransport in the skate (Raja erinacea) and shark (Squalus acanthias). Am J Physiol 273: R134–R142

Kissei Pharmaceutical Co. Ltd. (2009) Discontinuation of the development of "Remogliflozin" by GlaxoSmithKline, News Release July 3

Kitabchi AE, Umpierrez GE, Murphy MB, Barrett EJ, Kreisberg RA, Malone JI, Wall BM (2001) Management of hyperglycemic crises in patients with diabetes. Diab Care 24:131–153

Kleta R, Stuart C, Gill FA, Gahl WA (2004) Renal glucosuria due to SGLT2 mutations. Mol Genet Metab 82:56–58

Landsdell MI, Burring DJ, Hepworth D, Strawbridge M, Graham E, Guyot T, Betson MS, Hart JD (2008) Design and synthesis of fluorescent SGLT2 inhibitors. Bioorg Med Chem Lett 18:4944–4947

Li JM, Che CT, Lau CBS, Leung PS, Cheng CHK (2007) Desoxyrhaponticin (3, 5-dihydroxy-4'-methoxystilbene 3-O-beta-D-glucoside) inhibits glucose uptake in the intestine and kidney: In vitro and in vivo studies. J Pharmacol Exp Ther 320:38–46

Lin JT, Kormanec J, Wehner F, Wielert-Badt S, Kinne RKH (1998) High-level expression of Na+/D-glucose cotransporter (SGLT1) in a stably transfected Chinese hamster ovary cell line. Biochim Biophys Acta: Biomembr 1373:309–320

Meng W, Ellsworth BA, Nirschl AA, McCann PJ, Patel M, Girotra RN, Wu G, Sher PM, Morrison EP, Biller SA, Zahler R, Deshpande PP, Pullockaran A, Hagan DL, Morgan N, Taylor JR, Obermeier MT, Humphreys WG, Khanna A, Discenza L, Robertson JG, Wang A, Han S, Wetterau JR, Janovitz EB, Flint OP, Whaley JM, Washburn WN (2008) Discovery of dapagliflozin: a potent, selective renal sodium-dependent glucose cotransporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes. J Med Chem 51:1145–1149

Morrison AI, Panayotova-Heiermann M, Feigl G, Scholermann B, Kinne RKH (1991) Sequence comparison of the sodium-D-glucose cotransport systems in rabbit renal and intestinal epithelia. Biochim Biophys Acta 1089:121–123

Murer H, Hopfer U, Kinne-Saffran E, Kinne R (1974) Glucose transport in isolated brush-border and lateral-basal plasma-membrane vesicles from intestinal epithelial cells. Biochim Biophys Acta 345:170–179

Neumiller JJ, White JR Jr, Campbell RK (2010) Sodium-glucose co-transport inhibitors: progress and therapeutic potential in type 2 diabetes mellitus. Drugs 70:377–385

Nomura S, Sakamaki S, Hongu M, Kawanishi E, Koga Y, Sakamoto T, Yamamoto Y, Ueta K, Kimata H, Nakayama K, Tsuda-Tsukimoto M (2010) Discovery of canagliflozin, a novel C-glucoside with thiophene ring, as sodium-dependent glucose cotransporter 2 inhibitor for the treatment of type 2 diabetes mellitus. J Med Chem 53:6355–6360

Novakova R, Homerova D, Kinne RK, Kinne-Saffran E, Lin JT (2001) Identification of a region critically involved in the interaction of phlorizin with the rabbit sodium-D-glucose cotransporter SGLT1. J Membr Biol 184:55–60

Ohsumi K, Matsueda H, Hatanaka T, Hirama R, Umemura T, Oonuki A, Ishida N, Kageyama Y, Maezono K, Kondo N (2003) Pyrazole-O-glucosides as novel Na^+-glucose cotransporter (SGLT) inhibitors. Bioorg Med Chem Lett 13:2269–2272

Oku A, Ueta K, Arakawa K, Ishihara T, Nawano M, Kuronuma Y, Matsumoto M, Saito A, Tsujihara K, Anai M, Asano T, Kanai Y, Endou H (1999) T-1095, an inhibitor of renal Na+-glucose cotransporters, may provide a novel approach to treating diabetes. Diabetes 48:1794–1800

Oku A, Ueta K, Arakawa K, Kano-Ishihara T, Matsumoto M, Adachi T, Yasuda K, Tsuda K, Saito A (2000a) Antihyperglycemic effect of T-1095 via inhibition of renal Na+-glucose cotransporters in streptozotocin-induced diabetic rats. Biol Pharm Bull 23:1434–1437

Oku A, Ueta K, Arakawa K, Kano-Ishihara T, Matsumoto T, Adachi T, Yasuda K, Tsuda K, Ikezawa K, Saito A (2000b) Correction of hyperglycemia and insulin sensitivity by T-1095, an inhibitor of renal Na+-glucose cotransporters, in streptozotocin-induced diabetic rats. Jpn J Pharmacol 84:351–354

Oku A, Ueta K, Nawano M, Arakawa K, Kano-Ishihara T, Matsumoto M, Saito A, Tsujihara K, Anai M, Asano T (2000c) Antidiabetic effect of T-1095, an inhibitor of Na+-glucose cotransporter, in neonatally streptozotocin-treated rats. Eur J Pharmacol 391:183–192

Pajor AM, Randolph KM, Kerner SA, Smith CD (2008) Inhibitor binding in the human renal low- and high-affinity Na+/glucose cotransporters. J Pharmacol Exp Ther 324:985–991

Parent L, Supplisson S, Loo DDF, Wright EM (1992a) Electrogenic properties of the cloned Na^+ glucose cotransporter. 1. Voltage-clamp studies. J Membr Biol 125:49–62

Parent L, Supplisson S, Loo DDF, Wright EM (1992b) Electrogenic properties of the cloned Na+ glucose cotransporter. 2. A transport model under nonrapid equilibrium conditions. J Membr Biol 125:63–79

Patterson JE, Andriole VT (1995) Bacterial urinary-tract infections in diabetes. Infect Dis Clin North Am 9:25–51

Puntheeranurak T, Wildling L, Gruber HJ, Kinne RK, Hinterdorfer P (2006) Ligands on the string: single-molecule AFM studies on the interaction of antibodies and substrates with the Na+-glucose co-transporter SGLT1 in living cells. J Cell Sci 119:2960–2967

Puntheeranurak T, Kasch M, Xia X, Hinterdorfer P, Kinne RK (2007a) Three surface subdomains form the vestibule of the Na^+/glucose cotransporter SGLT1. J Biol Chem 282:25222–25230

Puntheeranurak T, Wimmer B, Castaneda F, Gruber HJ, Hinterdorfer P, Kinne RKH (2007b) Substrate specificity of sugar transport by rabbit SGLT1: single molecule AFM versus transport. Biochemistry 46:2797–2804

Raja MM, Tyagi NK, Kinne RKH (2003) Phlorizin recognition in a C-terminal fragment of SGLT1 studied by tryptophan scanning and affinity labeling. J Biol Chem 278:49154–49163

Sakhrani LM, Badiedezfooly B, Trizna W, Mikhail N, Lowe AG, Taub M, Fine LG (1984) Transport and metabolism of glucose by renal proximal tubular cells in primary culture. Am J Physiol 246:F757–F764

Scholl-Burgi S, Santer R, Ehrich JHH (2004) Long-term outcome of renal glucosuria type 0: the original patient and his natural history. Nephrol Dial Transplant 19:2394–2396

Stuart IS, Trayhurn P (2003) Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. Br J Nutr 89:3–9

Tsujihara K, Hongu M, Saito K, Inamasu M, Arakawa K, Oku A, Matsumoto M (1996) Na+-glucose cotransporter inhibitors as antidiabetics. 1. Synthesis and pharmacological properties of 4'-Dehydroxyphlorizin derivatives based on a new concept. Chem Pharm Bull 44:1174–1180

Tsujihara K, Hongu M, Saito K, Kawanishi H, Kuriyama K, Matsumoto H, Oku A, Ueta K, Tsuda M, Saito A (1999) Na+-glucose cotransporter (SGLT) inhibitors as antidiabetic agents. 4. Synthesis and pharmacological properties of 4'-dehydroxyphlorizin derivatives substituted on the B ring. J Med Chem 42:5311–5324

Tyagi NK, Goyal P, Kumar A, Pandey D, Siess W, Kinne RK (2005) High-yield functional expression of human sodium-D-glucose cotransporter1 in Pichia pastoris and characterization of ligand-induced conformational changes as studied by tryptophan fluorescence. Biochemistry 44:15514–15524

Tyagi NK, Kumar A, Goyal P, Pandey D, Siess W, Kinne RK (2007) D-glucose-recognition and phlorizin-binding sites in human sodium-D-glucose cotransporter 1 (hSGLT1): a tryptophan scanning study. Biochemistry 46:13616–13628

Tyagi NK, Puntheeranurak T, Raja M, Kumar A, Wimmer B, Neundlinger I, Gruber H, Hinterdorfer P, Kinne RK (2011) A biophysical glance at the outer surface of the membrane transporter SGLT1. Biochim Biophys Acta 1808(1):1–18, Epub 2010 Aug 6

Ueta K, Ishihara T, Matsumoto Y, Oku A, Nawano M, Fujita T, Saito A, Arakawa K (2005) Long-term treatment with the Na+-glucose cotransporter inhibitor T-1095 causes sustained improvement in hyperglycemia and prevents diabetic neuropathy in Goto-Kakizaki Rats. Life Sci 76:2655–2668

Ueta K, Yoneda H, Oku A, Nishiyama S, Saito A, Arakawa K (2006) Reduction of renal transport maximum for glucose by inhibition of Na+-glucose cotransporter suppresses blood glucose elevation in dogs. Biol Pharm Bull 29:114–118

van den Heuvel LP, Assink K, Willemsen M, Monnens L (2002) Autosomal recessive renal glucosuria attributable to a mutation in the sodium glucose cotransporter (SGLT2). Hum Genet 111:544–547

Venkatraman R, Singhi SC (2006) Hyperglycemic hyperosmolar nonketotic syndrome. Indian J Pediatr 73:55–60

Vick H, Diedrich DF, Baumann K (1973) Reevaluation of renal tubular glucose transport inhibition by phlorizin analogs. Am J Physiol 224:552–557

Washburn WN (2009) Evolution of sodium glucose co-transporter 2 inhibitors as anti-diabetic agents. Expert Opin Ther Pat 19:1485–1499

Weinglass AB, Swensen AM, Liu J, Schmalhofer W, Thomas A, Williams B, Ross L, Hashizume K, Kohler M, Kaczorowski GJ, Garcia ML (2008) A high-capacity membrane potential FRET-based assay for the sodium-coupled glucose co-transporter SGLT1. Assay Drug Dev Technol 6:255–262

Wielert-Badt S, Lin JT, Lorenz M, Fritz S, Kinne RK (2000) Probing the conformation of the sugar transport inhibitor phlorizin by 2D-NMR, molecular dynamics studies, and pharmacophore analysis. J Med Chem 43:1692–1698

Wright EM, Hirayama BA, Loo DF (2007) Active sugar transport in health and disease. J Intern Med 261:32–43

Zhang XY, Urbanski M, Patel M, Cox GG, Zeck RE, Bian HY, Conway BR, Beavers MP, Rybczynski PJ, Demarest KT (2006) Indole-glucosides as novel sodium glucose co-transporter 2 (SGLT2) inhibitors. Part 2. Bioorg Med Chem Lett 16:1696–1701