Phlorizin Pretreatment Reduces Acute Renal Toxicity in a Mouse Model for Diabetic Nephropathy*5

Received for publication, March 15, 2013, and in revised form, August 8, 2013 Published, JBC Papers in Press, August 11, 2013, DOI 10.1074/jbc.M113.469486

Bas Brouwers1, Vincent P. E. G. Pruniau1, Elisa J. G. Cauwelier4, Frans Schuit5, Evelyne Lerut*, Nadine Ectors*, Jeroen Declercq4,2,3, and John W. M. Creemers2

From the 1Laboratory for Biochemical Neuroendocrinology, Department of Human Genetics, and the 2Department of Molecular Cell Biology, KU Leuven, 3000 Leuven and the 4Department of Pathology, University Hospital Gasthuisberg, 3000 Leuven, Belgium

Background: The diabetogenic agent streptozotocin (STZ) induces direct kidney injury, which is a setback in diabetic nephropathy (DN) research.

Results: The Sglt inhibitor phlorizin reduces STZ uptake and hence toxicity in the kidneys.

Conclusion: In the kidney, STZ toxicity is mediated by Sglt.

Significance: Using the proposed STZ regimen, researchers can now induce DN without direct damage to proximal tubuli.

Phlorizin pretreatment reduces acute renal toxicity in a mouse model for diabetic nephropathy.

Streptozotocin (STZ)4 is widely used as diabetogenic agent in animal models for diabetic nephropathy (DN). However, it is also directly cytotoxic to kidneys, making it difficult to distinguish between DN-related and STZ-induced nephropathy. Therefore, an improved protocol to generate mice for DN studies, with a quick and robust achievement of the diabetic state, without direct kidney toxicity is required. To investigate the mechanism leading to STZ-induced nephropathy, kidney damage was induced with a high dose of STZ. This resulted in delayed gastric emptying, at least partially caused by impaired desacyl ghrelin clearance. STZ uptake in the kidneys is to a large extent mediated by the sodium/glucose cotransporters (Sglt) because the Sglt inhibitor phlorizin could reduce STZ uptake in the kidneys. Consequently, the direct toxic effects in the kidney and the gastric dilatation were resolved without interfering with the β-cell toxicity. Furthermore, pancreatic STZ uptake was increased, hereby decreasing the threshold for β-cell toxicity, allowing for single low non-nephrotoxic STZ doses (70 mg/kg).

In conclusion, this study provides novel insights into the mechanism of STZ toxicity in kidneys and suggests a more efficient regime to induce DN with little or no toxic side effects.

Streptozotocin (STZ)4 is a glucose analog commonly used as a diabetogenic agent in experimental models of diabetic nephropathy (DN) (1–3). STZ is an alkylating agent of the nitrosourea class causing DNA damage and cell death. It is efficiently taken up by β-cells via the glucose transporter Glut2, leading to diabetes by β-cell destruction (1–4). However, besides β-cells, other tissues such as the kidney are also susceptible to STZ toxicity (5, 6), making it difficult to distinguish between DN-related events and direct effects of STZ in these organs. In this regard, single high doses of STZ have a nonspecific cytotoxic effect that causes acute kidney damage in rodents (7–9). In addition, STZ induces direct damage to the liver, causing lipid peroxidation, mitochondrial swelling, and peroxisome proliferation, which occur before the onset of hyperglycemia (10). STZ is also immunosuppressive in vitro and in vivo (11) and directly toxic to skeletal muscle myoblasts (12) and cardiomyocytes (13), which is presumably independent of Glut2, as this transporter is not expressed in these cell types (14, 15). To overcome direct STZ-mediated effects to the kidneys in mouse models of DN, the Animal Models of Diabetic Complications Consortium (AMDCC) recommends multiple low doses of STZ over single high dose regimens (reviewed in Ref. 2). However, studies using these multiple low dose protocols show that the diabetes incidence rate with non-fasting blood glucose levels ≥400 mg/dl after 3 weeks is only 50% in C57BL/6 mice (3). Therefore, there is a clear need for optimization of the STZ protocols to generate mouse models for DN, with a quicker achievement of the diabetic state, higher diabetes incidence rates, and most importantly, absence of direct toxicity to the kidneys. To address this challenge, a single high dose STZ regimen was used to characterize the mechanism of STZ-mediated kidney injury. Furthermore, we present a new model for STZ-induced DN, by pretreating mice with the non-specific sodium-glucose cotransporter (Sglt) inhibitor phlorizin, a natural product found in a number of fruit trees (16). This modification results in a quick and robust induction of hyperglycemia by using a single low dose of STZ that does not induce direct renal damage.

EXPERIMENTAL PROCEDURES

Mice—Experiments were conducted in 12-week-old male C57BL/6J (Janvier, Le Genest-Saint-Isle, France), according to...
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The guidelines approved by the KU Leuven Ethical Committee. After overnight fasting, mice were injected intraperitoneally with 125, 200, or 250 mg/kg of bodyweight STZ (Sigma) freshly dissolved in 0.05 M sodium citrate buffer, pH 4.5. Blood glucose levels were assessed daily at 9 a.m. using a Contour glucose meter (Bayer, Leverkusen, Germany) via tail puncture. Mice were sacrificed by cervical dislocation 3 days after injection with STZ or vehicle. Tissues from the hypothalamus, kidneys, liver, and stomach were collected for histological analysis and RNA isolation. The relative stomach weight was determined by dividing the stomach weight after dissection at the pylorus and the gastroesophageal junction by the total body weight. To block Sglt1 and Sglt2 in the kidney, mice were injected with phlorizin. Because phlorizin is injected subcutaneously while STZ is injected intraperitoneally, pretreatment with phlorizin is required before STZ injection. Here, mice were injected twice subcutaneously with 400 mg/kg of phlorizin (Sigma; dissolved in 10% EtOH, 15% dimethyl sulfoxide and 75% normal saline (0.9% w/v NaCl)) 16 and 2 h prior to STZ administration.

Histology—Mouse tissues were fixed in 4% formaldehyde in PBS (Invitrogen), embedded in paraffin, and sectioned at 5-μm thickness. Sections were stained with Gill’s hematoxylin and eosin to determine overall histology according to standard procedures. Glut2 staining was performed on kidney sections. Rehydrated sections were heated for 20 min in Target Retrieval Solution (pH 6.1, Dako, Glostrup, Denmark). After blocking with 20% normal goat serum (Dako) in PBS, slides were incubated for 2 h with polyclonal rabbit anti-Glut2 antibody (1:5000, Millipore, Billerica, MA) diluted in Dako antibody diluent followed by rabbit EnVision (Dako) for 1 h. 3'-3-Diaminobenzidine (DAB+, Dako) was used as substrate chromogen. Overall histology and Glut2-positive tubular cells were evaluated for each section using a Leica DM 2500 M upright microscope (Leica, Solms, Germany).

Quantitative Real-time PCR—Total RNA was isolated using Nucleospin RNA II (Macherey Nagel, Düren, Germany) according to the manufacturer’s protocol. First strand cDNA was synthesized using iScript Select cDNA synthesis kit (Bio-Rad) using random hexamers. Primers were designed with the ProbeFinder software (Roche Applied Science, Basel, Switzerland, listed in supplemental Table S1). Quantitative real-time PCR (qRT-PCR) was performed in triplicate with MyiQ single-color real-time PCR detection system (Bio-Rad) using SYBR Green. Samples were normalized to glyceraldehyde 3-phosphate dehydrogenase (Gapdh).

Measurement of Plasma Creatinine, Urea, and Desacyl Ghrelin Levels—Blood was taken from the tail vein for analysis of kidney function parameters. Plasma creatinine and urea levels were determined using the QuantiChrom creatinine assay kit (BioAssay Systems, Hayward, CA) and the urea assay kit (Abcam, Cambridge, UK), respectively, according to the manufacturer’s protocol. Plasma desacyl ghrelin concentrations were measured using a desacyl ghrelin ELISA kit (Gentaur, Paris, France).

STZ Uptake in Vivo—Twelve-week-old C57Bl/6J mice were fasted overnight and injected subcutaneously with 400 mg/kg of phlorizin or buffer alone 16 and 2 h prior to STZ administration. Total kidney and pancreas were isolated 20 min after STZ injection and homogenized with an Ultra-Turrax disperser (IKA, Staufen, Germany) in 2 ml of 6% perchloric acid. The lysate was centrifuged at 3,500 rpm, and STZ content in the supernatant was quantified as described in Ref. 17. Briefly, 0.2 ml of the supernatant was mixed with 0.5 ml of color reagent (0.5% sulfanilic acid, 0.2% [N-1-naphthyl]ethylenediamine dihydrochloride in 30% acetic acid), and 0.1 ml of 6 N HCl. The mixture was incubated at 60 °C for 10 min, and the absorbance at 595 nm was determined with a Victor X3 plate reader (PerkinElmer). Absorbance values were converted to μg of STZ by fitting to the standard curve prepared with different concentrations of STZ in acetic buffer (0.1 M acetic acid, 0.02 M sodium acetate, pH 4.0).

Statistical Analysis—Results were expressed as means ± S.E. Statistical analysis was performed with the unpaired Student’s t test or one-way ANOVA with Bonferroni’s correction. The follow-up of body weight and blood glucose was analyzed using repeated measures ANOVA. The significance level was set at p < 0.05.

RESULTS

High STZ Doses Cause Mislocalization and Partial Loss of Glut2 Expression in Kidney Proximal Tubules, Up-regulation of Acute Kidney Injury Markers, and Acute Tubular Necrosis—To characterize direct renal toxicity of STZ, 12-week-old C57BL/6J males were divided in different STZ dosage groups. STZ toxicity to kidneys is known to occur in Glut2-expressing cells in the S1 segment of kidney proximal tubule (5). Average kidney Glut2 expression tended to be higher in 125 and 200 mg/kg STZ-treated animals, but was not significantly different from control (Fig. 1a). However, expression levels in the highest dosage group decreased drastically to 20.7 ± 8.5% of control values (p < 0.01), indicating a loss in Glut2-positive cells. Likewise, the expression of Sglt2, which is expressed exclusively near the early proximal convoluted tubule (term S1) (18), also decreased significantly after treatment with 250 mg/kg of STZ (Fig. 1a). In contrast, the expression of Sglt1, which is expressed near the medullary proximal tubule (term S3) (18), remained unaltered after STZ treatment (Fig. 1a). This indicates a loss of the early proximal convoluted tubule expressing Glut2 and Sglt2, but not of the medullary proximal tubule expressing Sglt1. Immunohistochemistry for Glut2 on kidney slides (Fig. 1b) showed partial loss of cylindrical tubule cells and desquamation, indicating necrosis of Glut2-expressing cells in the highest dose group. In rare cases, non-necrotic cells with a basolateral shift of the Glut2 expression pattern were also observed. Overall, the Glut2 signal in this dosage group was much lower as compared with control. No abnormalities were observed for 125 and 200 mg/kg of STZ. To evaluate the presence of acute kidney injury, the expression of physiological markers for acute kidney injury was quantified by qRT-PCR (Fig. 1c). Neutrophil gelatinase-associated lipocalin (Ngal) expression in total kidney samples increased 4.3-fold in 125 mg/kg (p < 0.05) and 27.4-fold in 200 mg/kg (p < 0.001), but was the highest in the 250 mg/kg dosage group (863.2-fold increase as compared with control, p < 0.001). Kidney injury molecule-1 (Kim-1) expression increased 4.7-fold in 125 mg/kg (p < 0.01) and 96.1-fold in 200 mg/kg (p < 0.01), with the highest expression observed in...
the 250 mg/kg dosage group (515.5-fold as compared with control, \(p < 0.001\)) (Fig. 1c). To confirm acute kidney injury histologically, standard hematoxylin and eosin staining was performed (Fig. 1d). Kidneys in the highest dose group showed occasional loss of nuclei in proximal tubule cells and disruption of the brush border, suggesting at least moderate acute tubular injury. Dilatation of non-proximal tubules was observed. These data suggest severe kidney damage in the highest dosage group, caused by direct STZ toxicity.

C57BL/6J Mice Show an Enlarged Stomach after a Single High Dose of STZ—As shown in Fig. 2a, there was a variable increase in blood glucose levels depending on the administered STZ dose, which differed significantly 1 day after injection between each dosage group (125 mg/kg versus 200 mg/kg, \(p < 0.01\); 125 mg/kg versus 250 mg/kg, \(p < 0.005\); 200 mg/kg versus 250 mg/kg, \(p < 0.001\)). A sudden drop in blood glucose (Fig. 2a) and total body weight (supplemental Fig. S1a) was observed for the highest dosage group (250 mg/kg of STZ) after 3 days (\(p < 0.05\)). At this time point, mice that were administered the highest dose were hunched up and appeared to be in poor health. Mice were sacrificed, and stomachs were dissected and weighed. Mice in the highest STZ dose group showed extensive gastric dilatation after sacrifice (Fig. 2b). The stomach weight relative to total body weight was significantly increased after injection with the high dose of STZ (Fig. 2c). The increased stomach weight was not caused by an increased food intake as the food intake in mice injected with high dose of STZ was significantly reduced 1 and 2 days after STZ injection (supplemental Fig. S1b). Likewise, the water intake was also significantly reduced 1 day after STZ injection (supplemental Fig. S1c). Nevertheless, as onset of diabetes is characterized by thirst, a significant increase of the water intake was observed 3 days after STZ injection. It is unlikely that this would explain the gastric dilation because excessive urination was also observed. Mouse stomachs of the highest dose group showed no gross histological abnormalities, and expression of endocrine effectors was unaltered, ruling out a direct effect of STZ on the stomach (supplemental Fig. S2). Direct toxicity to Glut2-expressing hypothalamic neurons is also unlikely to explain the gastric dilatation because STZ itself is unable to cross the blood-brain barrier (19). Indeed, there was no reduction observed in hypothalamic Glut2 expression (supplemental Fig. S3). However, expression of orexigenic Agrp and anorexigenic Pomc increased and decreased, respectively, but there was no difference between the lowest and the highest STZ dose, marking this as a mere effect of the diabetic state (20, 21). STZ-
induced liver injury has been described before (10, 22), both in the subacute and in the acute phase of toxicity, hereby distinguishing direct effects from hyperglycemia-related changes. Livers from mice in different treatment groups showed a dose-dependent decrease in Glut2 expression (supplemental Fig. S4), indicating that direct STZ-induced liver toxicity is most likely mediated through Glut2. Consistent with these findings, we observed dose-dependent liver damage, as evidenced by progressive foaming of the cytoplasm, more frequently in hepatocytes in the vicinity of central veins. However, not all the mice in the highest dose group showed liver injury to the same extent, which was in some cases not even detectable via standard H&E staining. Thus, changes in the liver cannot account for the observed stomach phenotype. Because decreased stomach motility is common in patients with end-stage kidney disease (23–25) and in nephrectomized rats (26), impaired kidney function as a result of direct STZ toxicity was further investigated as a cause of this stomach phenotype.

Kidney Function Is Impaired in Mice Treated with High Doses of STZ—To assess kidney function, blood plasma was collected 3 days after STZ administration, and kidney function parameters were quantified. Blood urea nitrogen (BUN) increased to 52.9 mg/dl in mice treated with 250 mg/kg of STZ as compared with 27.2 mg/dl in controls (p < 0.001), whereas plasma creatinine levels rose to 0.48 mg/dl (controls, 0.33 g/dl; p < 0.01), demonstrating kidney damage in the 250 mg/kg STZ-treated group (Fig. 3a). Plasma desacyl ghrelin levels are increased in end-stage kidney disease patients and nephrectomized rats, suggesting that the kidney is an important site for clearance and/or degradation of this hormone (27–29). Plasma desacyl ghrelin levels were significantly higher in the 250 mg/kg STZ-treated group as compared with vehicle-treated mice (3547 ± 269 fmol/ml versus 849 ± 183 fmol/mL, p < 0.001) (Fig. 3b). These data indicate a substantial impairment of kidney function in the highest STZ dosage group. It has been described that accumulation of desacyl ghrelin impairs gastric motility in mice (30), which would explain the disturbed gastric motility at least partially in the 250 mg/kg STZ-treated group.

Pretreatment with the Sglt1/Sglt2 Inhibitor Phlorizin Causes a Decrease in Acute Kidney Injury Parameters and a Rescue of the Stomach Phenotype—Sodium-dependent glucose cotransporters Sglt1 and Sglt2 are expressed in the proximal tubule of the nephron and contribute to renal glucose reabsorption (31). Because Sglt1 and Sglt2 are in direct contact with the blood filtrate, we hypothesized that administered STZ could be taken up, at least partially, by these cotransporters due to its glucose moiety, resulting in toxicity to proximal tubule cells. To test this hypothesis and to investigate whether or not the kidney damage is a causative factor of the reduced gastric emptying, mice were pretreated with phlorizin, a competitive Sglt inhibitor that does not inhibit Glut2 (31) (Fig. 4). This would block STZ transport through Sglt1 and Sglt2 and as such reduce STZ toxicity in the kidney. If kidney damage is the causative factor for the delayed gastric emptying, the stomach phenotype should be rescued as well. Indeed, pretreatment with 400 mg/kg of phlorizin 2 h before administration of 250 mg/kg of STZ prevented the stomach enlargement completely (p < 0.01) (Fig. 4a). Furthermore, loss of Glut2 expression caused by injection with 250 mg/kg of STZ was also rescued by pretreatment with phlorizin (250 mg/kg of STZ, 18 ± 3.5% of control; 250 mg/kg of STZ + phlorizin, 80 ± 11.3%, not significantly different from control) (Fig. 4b). Ngal and Kim-1 expression was significantly reduced in the phlorizin group as compared with the group treated with 250 mg/kg of STZ + buffer alone (Fig. 4b). Proximal tubule cells were more eosinophilic in the phlorizin group, and loss of nuclei and disruption of brush-border membrane were less apparent as compared with treatment with buffer alone (Fig. 4, c and d). These data suggest at least a partial rescue of the stomach phenotype by inhibiting STZ entry in proximal tubule...
cells. Sglt1 is not expressed in the stomach and hypothalamus and only expressed at low levels in the liver (32). Sglt2 is highly expressed in the kidney but not in the stomach, liver, and hypothalamus (supplemental Fig. S5). As such, this experiment strongly suggests that the kidney damage is the causative factor of the reduced gastric motility.

Phlorizin Pretreatment Inhibits STZ Uptake in Kidneys and Increases STZ Uptake in Pancreas —To further substantiate the hypothesis that STZ can enter tubular cells via Sglts, in vivo STZ uptake experiments were performed with or without phlorizin pretreatment (Fig. 5). Kidney STZ content was significantly lower in the phlorizin group 20 min after STZ injection (Fig. 5a; 19.6 ± 0.9 μg of STZ per total kidney versus 29.7 ± 2.5 μg for the control group, p < 0.01), indicating inhibition of STZ entry in tubular cells. In contrast, uptake in the pancreas was significantly increased after phlorizin pretreatment (21.4 ± 1.7 μg of STZ per total pancreas versus 11.8 ± 2 μg for controls, p < 0.01). This suggests that phlorizin inhibits the uptake of STZ in the kidney and increased uptake in the pancreas. Blood glucose measured 2 h after phlorizin pretreatment was significantly reduced as compared with pretreatment with buffer alone (Fig. 5b). This is consistent with the established blood glucose-lowering effect of phlorizin due to blocked reabsorption of the filtered glucose in the kidney.

Phlorizin Pretreatment Allows for Lower STZ Dosages and Minimizes Direct Kidney Damage —The observation that phlorizin pretreatment increased STZ uptake in the pancreas prompted us to reassess the minimal diabetogenic dose. Phlorizin lowered the threshold for STZ toxicity in β-cells as 50% of the mice became diabetic after injection of 65 mg/kg of STZ...
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FIGURE 5. Influence of phlorizin on STZ uptake in pancreas and kidney. Total kidney and pancreas were isolated 20 min after 250 mg/kg of STZ with or without phlorizin pretreatment, and STZ content was quantitatively measured. a, STZ content in μg per total kidney or pancreas. Data are shown as means ± S.E., n = 5–6 per group, **, p < 0.01. b, blood glucose levels after an overnight fast, 2 h after the second pretreatment with phlorizin, shown as means ± S.E., n = 5–6 per group, **, p < 0.01.

FIGURE 6. Blood glucose levels, kidney gene expression analysis and BUN and creatinine levels after a single low dose of STZ. C57BL/6J mice were administered a single low dose of 70 mg/kg of STZ, with or without phlorizin pretreatment. a, changes in blood glucose levels, n = 3 per group. Values at time point 0 represent glucose levels just before STZ administration. b, kidney gene expression levels, 3 days after STZ injection. Data are shown as fold induction as compared with untreated control as means ± S.E., n = 3 per group; n.s., not significant. c, BUN and plasma creatinine levels were assayed 3 days after injection with 70 mg/kg of STZ with or without phlorizin pretreatment and in control mice. Data are presented as means ± S.E., n = 3–5 per group.

DISCUSSION

STZ is the most commonly used diabetogenic agent in diabetes research (4, 6). However, its toxicity to β-cells has been frequently reported to be accompanied by aspecific detrimental effects to other tissues, particularly in high dose treatment regimens (5, 8, 10, 11, 33). This phenomenon is well known and limits the use of the STZ-induced diabetic rodent model in DN research (1–3). Here, we aimed to characterize the mechanism by which STZ enters and subsequently damages kidney tubule cells by a single high dose. This allowed us to introduce an improved protocol, with 100% efficiency, but with the absence of unwanted side effects. Direct STZ damage to the kidneys is generally believed to be mediated by Glut2 uptake in proximal tubule cells, causing acute tubular necrosis and subsequent changes in kidney function (7). Nevertheless, given the basolateral location of Glut2 and its role in transporting glucose from the intracellular compartment of cells into the blood, it is unlikely that this transporter plays a primary role in proximal tubular uptake of STZ. After STZ injection, a decrease in Glut2 mRNA expression was observed, particularly in the highest dose group, suggesting a loss of Glut2-expressing proximal tubule cells. Indeed, on a histological level, there was a much weaker Glut2 signal in the highest dose group. Kidney Glut2 expression is increased in diabetic rats (34), suggesting that loss of Glut2 signal is caused by direct STZ toxicity and not by the diabetic state. Partial exfoliation of proximal tubule cells and perturbation of the brush-border membrane was observed, indicative of acute tubular necrosis and/or apoptosis (35). To further substantiate direct STZ-induced kidney damage, expression of Ngal and Kim-1, both valuable biomarkers for acute kidney injury in animal models and patients (36, 37), were investigated. Expression of both genes was vastly increased in the highest dose groups, at levels that highly surpassed those of lower STZ regimens. Similar increases in Kim-1 and Ngal expression were observed in ischemia-reperfusion injury, and both are the top dysregulated genes early and late after acute kidney injury (38). Impaired kidney function was reflected by increased plasma urea and creatinine levels. Strikingly, mice in the highest dose group suffered severe stomach bloating. This condition is similar to the gastric dilatation syndrome found in mice exposed to high levels of environmental antigens, which is associated with chronic nephropathy, hypergastrinemia, and gastritis (39). Moreover, previous work on STZ experiments in nonhuman primates revealed early lethality caused by gastric dilatation in some cases, and several interesting, yet inconclusive causative factors were postulated (40, 41). Three days after injection with the highest dose of STZ, mice exhibited a drop in blood glucose, probably reflecting impaired stomach emptying and a lack of adequate nutrient intake. In the same context, patients with gastric dysfunction caused by chronic renal disease have hypoalbuminemia, which is indicative of nutritional status and predictive for survival (42–44). Stomach motility is...
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tightly regulated by the enteric nervous system and gastric and extra-gastric endocrine mediators, with a prominent role for the hormone ghrelin (45–47). Ghrelin is a 28-amino acid peptide hormone originally isolated from the rat stomach as an endogenous ligand for the growth hormone secretagogue receptor (48). Its n-octanoylated form has a broad range of effects and, of particular interest, it promotes food intake and stimulates gastric motility (49, 50). However, desacyl ghrelin has a negative impact on energy balance, inhibiting food intake and gastric emptying (30). Studies in patients showed that total and desacyl ghrelin are increased in patients with end-stage renal disease (27–29), and a similar rise in plasma levels was observed in nephrectomized mice (27). This suggests that the kidneys have a role in the metabolism and/or clearance of ghrelin. Mice of the highest STZ dose group showed a 4.1-fold increase in desacyl ghrelin levels, suggestive of severely impaired renal clearance of the hormone. As desacyl ghrelin inhibits stomach motility in mice (30), this might, at least partially, explain the gastric dilatation. Lack of proper clearance of peptide hormones such as desacyl ghrelin might also be causative of the delayed gastric emptying frequently observed in chronic renal disease patients (23–25).

STZ has been shown to exert direct toxicity to certain types of immune cells (11), skeletal muscle myoblasts (12), and cardiomyocytes (13), although these cells do not express Glut2 at detectable levels. Thus, STZ uptake can be mediated through glucose transporters other than Glut2. In the kidney, sodium-glucose cotransporters Sglt1 and Sglt2 are expressed on the apical side of proximal tubule cells; Sglt2 reabsorbs the bulk of the filtered glucose, whereas Sglt1 transports the remainder (51). In contrast, the third member of the same gene family, Sglt3, does not transport glucose (52). Given the blood glucose-lowering effects of Sglt inhibitors such as phlorizin and related molecules, they are valuable diabetes therapeutics (31). Because of the apical expression of Sglt3, we hypothesized that STZ through its glucose moiety could be, at least partially, taken up by these transporters from the blood filtrate. To validate this hypothesis, the Sglt transporters were temporarily blocked with phlorizin. With phlorizin, it is possible to achieve a similar degree of glycemic control over a 12-h period to that accomplished with insulin therapy (53). This period should certainly be long enough to inhibit STZ uptake in the kidneys because STZ is rapidly cleared (54). Indeed, pretreatment with phlorizin just before STZ administration successfully reduced STZ uptake into proximal tubule cells. This partially rescued the observed kidney damage and prevented gastric dilatation. In contrast, STZ uptake in the pancreas was increased, most likely caused by the blood glucose-lowering effect of phlorizin. This reduces the competition between STZ and glucose for uptake through Glut2 in β cells. Indeed, by pretreating with phlorizin, we were able to induce diabetes with a single low STZ dose (70 mg/kg) that is non-diabetogenic under normal conditions. In this dose group, kidney damage was absent, most likely as a result of minimal renal STZ uptake and thus less direct effects. The blood glucose-lowering effect of phlorizin as the indirect cause of decreased renal STZ uptake is unlikely because this would imply less competition for Glut2 and hence increased STZ uptake in the kidneys.

In conclusion, we identify acute kidney damage as the cause of decreased gastric motility after single high dose STZ administration. Furthermore, this is to our knowledge the first study to show renal STZ uptake via Sglt transporters. Because pretreatment with the Sglt inhibitor phlorizin reduces renal damage while simultaneously increasing STZ uptake in pancreatic β cells, these results suggest that this combination regimen facilitates the use of lower concentrations of STZ without loss of diabetogenic effect, whereas further reducing toxic side effects to other tissues.

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