Selective sodium-dependent glucose transporter 1 inhibitors block glucose absorption and impair glucose-dependent insulinotropic peptide release

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Dobbins RL, Greenway FL, Chen L, Liu Y, Breed SL, Andrews SM, Wald JA, Walker A, Smith CD. Selective sodium-dependent glucose transporter 1 inhibitors block glucose absorption and impair glucose-dependent insulinotropic peptide release. Am J Physiol Gastrointest Liver Physiol 308: G946–G954, 2015. First published March 12, 2015; doi:10.1152/ajpgi.00286.2014.—GSK-1614235 and KGA-2727 are potent, selective inhibitors of the SGLT1 sodium-dependent glucose transporter. Nonclinical (KGA-2727) and clinical (GSK-1614235) trials assessed translation of SGLT1 inhibitor effects from rats to normal human physiology. In rats, KGA-2727 (0.1 mg/kg) or vehicle was given before oral administration of 3-0-methyl-α-D-glucopyranose (3-O-methylglucose, 3-OMG) containing 3-[3H]OMG tracer. Tracer absorption and distribution were assessed from plasma, urine, and fecal samples. SGLT1 inhibition reduced urine 3-OMG recovery and increased fecal excretion. SGLT1 inhibitor effects on plasma glucose, insulin, gastric inhibitory peptide (GIP), and glucagon-like peptide-1 (GLP-1) concentrations were also measured during a standard meal. Incremental glucose, insulin, and GIP concentrations were decreased, indicating downregulation of β-cell and K cell secretion. Minimal effects were observed in the secretion of the L cell product, GLP-1. With the use of a three-way, crossover design, 12 healthy human subjects received placebo or 20 mg GSK-1614235 immediately before or after a meal. Five minutes into the meal, 3-OMG was ingested. Postmeal dosing had little impact, yet premeal dosing delayed and reduced 3-OMG absorption, with an AUC0–10 of 231 ± 31 vs. 446 ± 31 μg·h⁻¹·ml⁻¹ for placebo. Recovery of tracer in urine was 1.2 ± 0.7 g for premeal dosing and 2.2 ± 0.1 g for placebo. Incremental concentrations of insulin, C-peptide, and GIP were reduced for 2 h with premeal GSK-1614235. Total GlP-1 concentrations were significantly increased, and a trend for increased peptide YY (PYY) was noted. SGLT1 inhibitors block intestinal glucose absorption and reduce GIP secretion in rats and humans, suggesting SGLT1 glucose transport is critical for GIP release. Conversely, GLP-1 and PYY secretion are enhanced by SGLT1 inhibition in humans.

GSK-1614235; KGA-2727; gastric inhibitory peptide; clinical trial

SODIUM-DEPENDENT GLUCOSE TRANSPORTER 1 (SGLT1; SLC5A1) is the primary pathway for intestinal absorption of glucose and galactose (12, 33, 36–38). Most prominently expressed in the small intestine and renal proximal tubule (1, 28), SGLT1 is also reportedly localized in circumvallate papillae (21), brain (24), and heart (2). In the gastrointestinal tract, this integral membrane protein is localized to the apical membrane of the enterocyte and enteroendocrine cells where it utilizes the energy of the inward-directed sodium gradient to drive concentrative monosaccharide uptake into cells (25, 27, 32, 35). In addition to its role in glucose absorption, SGLT1 could also be an important glucose sensor in the intestinal lumen that regulates enterocyte and enteroendocrine cell responses following ingestion of meals (22, 31, 39).

For patients with type 2 diabetes mellitus (T2DM), SGLT1 mRNA and protein expressions are increased (9), and SGLT1 inhibition is expected to reduce postprandial absorption of glucose and galactose. This would blunt the magnitude of postprandial glucose excursions, delay the systemic appearance of glucose after a meal, increase the intraluminal concentration of glucose, and expose a larger segment of the lower gastrointestinal tract to carbohydrate signals that can trigger release of enteroendocrine peptides (29). Because clinical evidence indicates that reducing postprandial glucose excursions and improving glycemic control can lower the risk of diabetic complications (3, 7, 23, 26), SGLT1 inhibition could provide a meaningful mechanism for the treatment of T2DM. Studies in animal models have demonstrated that the nonspecific SGLT transport inhibitor phlorizin blocks glucose absorption, reduces postprandial glucose area under the curve (AUC), and lowers fasting plasma glucose and hemoglobin A1c (8, 10, 20, 22). Interpretation of in vivo results is confounded because phlorizin is metabolized to produce phloretin, an active metabolite that blocks facilitated glucose (GLUT) transporters; facilitates diffusion of urea and glycerol; and inhibits membrane transport of chloride, bicarbonate, and lithium in mammalian erythrocytes (10). KGA-2727 is a potent and selective inhibitor of SGLT1. It has inhibition constant (Ki) values of 97 and 13,600 nM for human (h) SGLT1 and hSGLT2, respectively (29). KGA-2727 improved postprandial hyperglycemia in streptozotocin-induced diabetic rats, and chronic administration reduced glucose concentrations and glycated hemoglobin in Zucker diabetic fatty rats (29).

GSK-1614235 is an analog of KGA-2727 with Ki values of 27 and 8,170 nM for hSGLT1 and hSGLT2, respectively (4). It is a specific SGLT1 inhibitor compared with other molecules that primarily inhibit renal glucose reabsorption via SGLT2 (4) or have dual effects on SGLT1 and SGLT2 (40, 41). This report presents the first set of nonclinical and clinical studies that have determined the effects of selective SGLT1 inhibitors in animal models and confirmed their translation to normal human physiology.

MATERIALS AND METHODS

All nonclinical procedures were performed in compliance with the Animal Welfare Act and United States Department of Agriculture regulations and were approved by the GlaxoSmithKline Animal Care and Use Committee. The clinical trial (GlaxoSmithKline study identifier SGA112534; ClinicalTrials.gov identifier NCT00976261) was
conducted in healthy subjects at the Pennington Biomedical Research Center (Baton Rouge, LA) in accordance with good clinical practice guidelines and the principles of the Helsinki Declaration. The study protocol was approved by the Pennington Biomedical Research Center Institutional Review Board. Written informed consent was obtained from each subject.

Materials

KGA-2727, GSK-1614235 tablets, and placebo tablets were provided by Kissei Pharmaceuticals (Nagano, Japan). For placebo tablets pregelatinized starch was substituted for GSK-1614235. GSK-1614235 is also designated as KGA-3235 and DSP-3235 (Dainippon Sumitomo Pharma, Osaka, Japan), and the structure has been published (4). Unlabeled 3-O-methyl-α-D-glucopyranose (3-OMG) and 3-O-[1$^3$H-methyl]-α-D-glucose (sp act 80 Ci/mmol) were purchased from Sigma-Aldrich, (St. Louis, MO) and American Radiolabeled Chemicals (St. Louis, MO), respectively.

Nonclinical Studies

3-OMG absorption studies. Male Sprague-Dawley rats obtained from Charles River (Raleigh, NC) arrived at 7 wk of age and were housed under controlled conditions (12:12-h light-dark cycle, 24°C, and 50% relative humidity) with free access to rodent food (Purina 5001; Harlan Teklad, Indianapolis, IN). After a 1-wk acclimation period, rats were gavaged with isoflurane (Abbott Laboratories), and jugular and bladder cannulation surgery was performed. After 1 wk of recovery, the rats were randomized to two groups, vehicle and KGA-2727, and fasted overnight in metabolic cages. Baseline blood and urine samples were obtained followed by vehicle (0.5% hydroxypropylmethylcellulose/0.1% Tween 80, 5 ml/kg) or KGA-2727 (0.1 mg/kg dissolved in 5 mg/kg vehicle) treatment via oral gavage. Fifteen minutes after drug treatment, all rats were gavaged with a mixture of 3-$^3$H]OMG (10 μCi/kg) and unlabeled 3-OMG (0.8 g/kg). Blood and urine samples were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, and 24 h. After urine volume was recorded, radioactivity within 0.2 ml of plasma and 0.1 ml of urine was measured using a liquid scintillation counter. One hour after 3-OMG administration, rats were allowed to have free access to food and water for the rest of the study. Total fecal samples were collected at 6, 24, and 48 h in glass bottles, and Soluene-350 (PerkinElmer, Waltham, MA) was added at 5 ml/g feces. Fecal samples were then incubated in a shaker at 60°C over night. An aliquot of 1 ml of the extraction was mixed with 1 ml of Soluene-350 and incubated at 60°C for another hour. An aliquot of 0.5 ml of the final mixture was then mixed with 15 ml of Hionic-Fluor (PerkinElmer Life and Analytical Sciences, Shelton, CA). Sample radioactivity was then measured after temperature and light adaptation.

Calculation of total amount of 3-$^3$H]OMG in blood. The amount from each assay (in 0.2 ml of plasma) times the volume of plasma:

\[ \text{Plasma volume (ml) = blood volume (BV)/2;} \]

\[ \frac{BV (ml)}{body wt + 0.77}. \]

Meal test. Male Sprague-Dawley rats were fasted overnight (16 h) and randomly assigned to vehicle or KGA-2727 (0.1 mg/kg) groups. Compounds were administered via oral gavage (5 ml/kg volume), and regular chow food was provided 5 min later (0 min). Animals had free access to food and water during the rest of the study. Blood samples were taken from the jugular vein at 0, 5, 15, 30, 60, 120, 180, and 240 min, and plasma samples were saved for further analyses.

Assays. Plasma glucose was measured using the Olympus AU640 clinical chemistry analyzer (Beckman Coulter, Irving, TX). Insulin, gastric inhibitory peptide (GIP), and peptide YY (PYY) were assayed using a MILLIPLEX MAP kit (St. Charles, MO) and analyzed on a Luminex 100 (Austin, TX). Total glucagon-like peptide-1 (GLP-1) was assayed by the MSD Total GLP-1 assay kit (K150JVC-1) and analyzed on an MSD Sector Imager 6000 (Meso Scale Discovery, Gaithersburg, MD).

Clinical Studies

Study population. A total of 38 subjects were screened, and 12 were enrolled. Women of non-childbearing potential and men who were 18–65 yr of age with body mass index (BMI) 19–30 kg/m² could be enrolled. All subjects had to be in good general health without clinically significant cardiac, pulmonary, or renal disease; or positive tests for hepatitis B surface antigen, hepatitis C virus, or human immunodeficiency virus. Specific exclusion criteria included irritable bowel syndrome, gastrointestinal surgeries, lactose intolerance, or hepatobiliary conditions that could interfere with the absorption of GSK-1614235 or with the endpoints of the study.

Study procedures. The study consisted of three treatment periods with subjects randomized to receive matching placebo or 20 mg GSK-1614235 administered either immediately before breakfast or 30 min after the first bite of the meal. Subjects were admitted to the clinical research unit the evening before receiving study drug (day –1). Following dosing and blood sampling, subjects were discharged on day 3 after breakfast. Subjects received a standardized diet of 2,200 kcal/day with 25% of calories provided at breakfast, 35% at lunch, and 40% at supper. The macronutrient distribution was 45% of calories from carbohydrate, 35% from fat, and 20% from protein.
Breakfast on day 1 of each period was served with a solution of 3-OMG containing 3 g dissolved in tap water to a volume of 50 ml. The solution was swallowed by subjects ~5 min into eating breakfast, and the empty cup was rinsed with a second 50-ml aliquot of tap water that was ingested. Measurement of 3-OMG in plasma and urine is an established method for safe assessment of glucose absorption in clinical studies (13, 17, 18).

Body weight, height, and BMI were recorded at screening. Physical exam results, blood pressure, heart rate, and 12-lead electrocardiogram (ECG) recordings were recorded at screening, before dosing each treatment period, and at the follow-up visit. Hematology, clinical chemistry, and urinalysis assessments were performed at screening, before breakfast on days 1 and 3, and at the follow-up visit. Adverse events were collected by the site staff based on patient reports.

Fig. 2. Plasma concentrations of glucose (A), insulin (B), glucose-dependent insulino tropic polypeptide (C), peptide YY (D), and total glucagon-like peptide-1 (E) in normal rats. The bar graphs represent change in concentration relative to the baseline obtained before administration of the KGA-2727. A mixed meal of standard chow was provided 5 min after administration of 0.1 mg/kg KGA-2727 or dilution vehicle (0.5% HPMC/0.1% Tween 80, 5 ml/kg). GIP, gastric inhibitory peptide; PYY, peptide YY; tGLP-1, total glucagon-like peptide-1. Values are means ± SE. *P < 0.05, **P < 0.01, and ***P < 0.001.
Pharmacodynamic assessments. METABOLITE AND HORMONE PROFILES (DAY 1). Identical meals were provided during each treatment period starting with supper on day 1. Samples for analyses of glucose, 3-OMG, insulin, C-peptide, glucagon, total GLP-1, active GLP-1, PYY, and GIP concentrations were obtained at frequent intervals for 10 h after study drug. Glucose concentrations were analyzed using the YSI 2300 STAT Plus Analyzer (YSI Life Sciences, Yellow Springs, OH). Insulin and total GLP-1 [GLP-1(7–39), GLP-1(9–39), and GLP-1(7–39) amidase] concentrations were determined using the MSD duplex assay (K15160C; Meso Scale Discovery). MSD assay kits were also used for measurement of glucagon (K151HCC) and active GLP-1 (K151 HZC). Millipore human ELISA kits were used to measure C-peptide (EZHCP-20K), GIP (EZHGIP-54K), and PYY (EZHPYY66K; Millipore, St. Charles, MO).

3-OMG ABSORPTION STUDIES. At scheduled times, 2 ml of whole blood for 3-OMG analyses were collected in a K2 EDTA tube and centrifuged at 1,300 g for 10 min at ~4°C to obtain a plasma aliquot. Urine samples for 3-OMG analyses were collected over the intervals of 0–4, 4–8, 8–12, 12–16, and 16–24 h. At the end of each collection window, one 2-ml aliquot of urine was saved for analysis. Harvested plasma or urine was stored at −70°C pending transport and analysis. 3-OMG was extracted from human plasma or urine by protein precipitation with 25 ml of sample added to 200 ml of acetonitrile containing 3-O-[13C6]methylglucose as an internal standard. Samples were then derivatized by adding 15 ml of 0.1 M methoxyamine hydrochloride to 150 ml supernatant and warming at 50°C for 30 min. After cooling to room temperature, 50 ml of the derivatized sample was diluted with 200 ml of acetonitrile before being analyzed. The ultra performance liquid chromatography-MS/MS system used for analysis consisted of an amide column coupled with a TurboIonSpray interface and multiple-reaction monitoring. Methods were validated over a range of 1–1,000 μg/ml.

Pharmacokinetic assessments. Blood samples were collected at predose and up to 48 h postdose for the determination of plasma concentrations of GSK-1614235 and two inactive metabolites (GSK-2313533 and GSK-2313537). Pharmacokinetic (PK) analyses of plasma concentration-time data were conducted using noncompartmental analysis with WinNonlin Professional Edition version 4.1 (Pharsight, Mountain View, CA). The PK parameters determined for each analyte included the area under the plasma concentration vs. time curve (AUC) from time 0 to the last time point with measurable concentration (AUC_{0-last}) and AUC_{0–t}, maximum observed plasma concentration, maximum time (t_{max}), and half-time for GSK-1614235, GSK-1614235, GSK-2313533, and GSK-2313537 were extracted from 75 ml of human plasma by solid-phase extraction using isotopically labeled internal standards ([2H6]GSK-1614235, [3H6]GSK-2313533, and [3H6]GSK-2313537). Extracts were analyzed by HPLC/MS/MS using a TurboIonSpray interface and multiple-reaction monitoring. Methods were validated over a range of 0.02–20 ng/ml.

Statistical Analyses

All nonclinical studies were analyzed using unpaired Student’s t-test. P values <0.05 were considered to indicate a significant difference between treatment groups. The key clinical endpoints were the AUC for 3-OMG concentrations and the weight mean concentrations of other pharmacodynamic (PD) parameters during the postprandial period from 0 to 2 and 0 to 4 h. Weighted means over a specific time interval for glucose and other parameters were derived as the AUC divided by the observed length of the collection interval. The AUC was calculated using the linear trapezoid method. Derived PD parameters were separately analyzed using a mixed-effects model with fixed-effect terms for period and treatment. Subject was treated as a random effect in the model. Point estimates and their associated 95% confidence intervals (CI) were constructed for the differences between each active treatment and placebo (premeal dosing, placebo and postmeal dosing, placebo). A post hoc regression analysis of individual PD parameters vs. 3-OMG, expressed as the percentage of

Table 1. Baseline demographic characteristics of healthy volunteer study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>12</td>
</tr>
<tr>
<td>Age, yr</td>
<td>32.6 (12.3)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2 [17]</td>
</tr>
<tr>
<td>Female</td>
<td>10 [83]</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>3 [25]</td>
</tr>
<tr>
<td>White</td>
<td>9 [75]</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.71 (0.12)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.8 (11.3)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.2 (3.5)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>87.5 (7.0)</td>
</tr>
</tbody>
</table>

Data are presented as means (SD), with the exception of sex and race, which are presented as n [%]. BMI, body mass index.

Fig. 3. Plasma concentrations of glucose (A), insulin (B), and C-peptide (C) in healthy human subjects. Premeal indicates 20 mg of GSK-1614235 administered immediately before breakfast, and postmeal indicates administration 30 min after the start of the meal. The meal contained 3 g of the 3-OMG tracer dissolved in water. Values are means ± SE.
placebo response in weighted mean AUC_{2-10} h, was performed to evaluate the relationship between postprandial glucose absorption and concomitant changes in gastrointestinal or pancreatic peptides secretion. There were no adjustments for multiplicity.

**RESULTS**

**Nonclinical Results**

3-OHG absorption in normal rats. Orally administered 3-[\(^{1}H\)]OGH was quickly absorbed from the small intestine and reached a peak plasma level within 30 min (Fig. 1A). Pretreatment with KGA-2727 significantly delayed the appearance and reduced plasma levels of 3-OHG compared with the vehicle group in the first 2 h (Fig. 1A). Absorbed 3-OHG was rapidly cleared from the blood into urine (Fig. 1B) and reached a low-level steady plasma concentration in 4 h in the vehicle group (Fig. 1A). KGA-2727-treated animals had significantly lower cumulative urine 3-OHG (292 vs. vehicle: 10.6). KGA-2727-treated animals had significantly lower cumulative urine 3-OHG (292 vs. vehicle: 10.6) but dramatically higher fecal excretion of the labeled glucose compared with that of the vehicle-treated rats (Fig. 1C). The fact that 29% (95% CI 14–34%) of the administered 3-OHG was recovered from feces in the KGA-2727 group compared with only 4% (3–6%) in the vehicle group (Fig. 1D) strongly supported that KGA-2727 inhibited active transport of glucose in intestine. Total recovery of 3-OHG was lower for the KGA-2727-treated group.

Meal test. To examine the effect of KGA-2727 under normal feeding conditions, we performed a meal test in cannulated rats 5 min after a single dose of the compound. KGA-2727 did not impact food intake (KGA-2727: 9.3 vs. vehicle: 10.6) but dramatically higher fecal excretion of the labeled glucose compared with that of the vehicle-treated rats (Fig. 1C). The fact that 29% (95% CI 14–34%) of the administered 3-OHG was recovered from feces in the KGA-2727 group compared with only 4% (3–6%) in the vehicle group (Fig. 1D) strongly supported that KGA-2727 inhibited active transport of glucose in intestine. Total recovery of 3-OHG was lower for the KGA-2727-treated group.

**Clinical Results**

Safety. All subjects completed procedures as planned. Baseline clinical characteristics are listed in Table 1. For GSK-1614235 dosing before breakfast, the proportion of subjects reporting adverse events modestly increased (20 mg premeal: 4/12, 20 mg postmeal: 2/12, placebo: 2/12). Drug-related adverse events occurring with GSK-1614235 treatment were diarrhea (n = 4), abdominal pain (n = 1), and flatulence (n = 1). All were mild or moderate in intensity, and there were no serious adverse events. No apparent drug-related trends and no clinically relevant changes in clinical laboratory results, ECG measures, heart rate, or blood pressure were observed.

Pharmacodynamics. Single doses of GSK-1614235 administered at the start of breakfast or 30 min afterward yielded significant postprandial effects. Figure 3 depicts mean concentrations for glucose, insulin, and C-peptide for 4 h after breakfast, and Table 2 lists results of the statistical analyses for the 2- and 4-h postprandial period. No changes in weighted mean glucose concentrations were observed in either the premeal or the postmeal dosing groups. However, the peak incre-

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**Table 2. Summary of derived pharmacodynamic parameters**

<table>
<thead>
<tr>
<th>Glucose</th>
<th>2-Hour Postprandial</th>
<th>Postmeal (20 mg)</th>
<th>4-Hour Postprandial</th>
<th>Postmeal (20 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>mg/dl</td>
<td>93.5 ± 3.2</td>
<td>93.0 ± 3.2</td>
<td>94.4 ± 3.3</td>
<td>90.3 ± 2.4</td>
</tr>
<tr>
<td>Δ vs. pbo</td>
<td>-0.4 (5.2, 4.3)</td>
<td>1.0 (6, 5.9)</td>
<td>0.9 (2.3, 4.2)</td>
<td>0.4 (2.8, 3.7)</td>
</tr>
<tr>
<td>Insulin</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pmol/l</td>
<td>132 ± 14</td>
<td>84 ± 14</td>
<td>123 ± 14</td>
<td>96 ± 12</td>
</tr>
<tr>
<td>Δ vs. pbo</td>
<td>-48 (79, -17)</td>
<td>-9 (41, 22)</td>
<td>-16 (35, 4)</td>
<td>0 (20, 19)</td>
</tr>
<tr>
<td>C-peptide</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pmol/l</td>
<td>954 ± 82</td>
<td>694 ± 82</td>
<td>930 ± 85</td>
<td>823 ± 78</td>
</tr>
<tr>
<td>Δ vs. pbo</td>
<td>-260 (435, -85)</td>
<td>-14 (195, 167)</td>
<td>-86 (224, 51)</td>
<td>-13 (150, 124)</td>
</tr>
<tr>
<td>GIP</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pmol/l</td>
<td>30.6 ± 3.0</td>
<td>15.6 ± 3.4</td>
<td>27.1 ± 2.9</td>
<td>30.9 ± 2.7</td>
</tr>
<tr>
<td>Δ vs. pbo</td>
<td>-14.9 (20.9, -8.9)</td>
<td>-3.5 (8.7, 1.7)</td>
<td>-10.5 (15.1, -6.0)</td>
<td>-6.0 (10.5, -1.4)</td>
</tr>
<tr>
<td>GLP-1</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pmol/l</td>
<td>9.9 ± 1.1</td>
<td>13.7 ± 1.1</td>
<td>12.2 ± 1.1</td>
<td>10.3 ± 1.0</td>
</tr>
<tr>
<td>Δ vs. pbo</td>
<td>3.8 (1.9, 5.6)</td>
<td>2.2 (0.4, 4.1)</td>
<td>2.4 (0.4, 4.0)</td>
<td>2.1 (0.5, 3.6)</td>
</tr>
<tr>
<td>PYY</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pmol/l</td>
<td>34.2 ± 3.7</td>
<td>42.9 ± 4.7</td>
<td>35.9 ± 4.2</td>
<td>37.2 ± 4.0</td>
</tr>
<tr>
<td>Δ vs. pbo</td>
<td>8.7 (2.4, 19.7)</td>
<td>1.7 (8.6, 11.9)</td>
<td>7.3 (4.1, 18.6)</td>
<td>0.9 (10.6, 12.4)</td>
</tr>
</tbody>
</table>

Values are presented as adjusted means ± SE with differences (Δ) from placebo (pbo) presented as mean (95% confidence interval); n, no. of subjects. GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; PYY, peptide YY. Differences in sample size reflect missing samples that prevented calculation of the derived endpoint. Premeal indicates 20 mg of GSK-1614235 administered immediately before breakfast, and postmeal indicates administration 30 min after the start of the meal.
mental glucose value was reduced (−14 mg/dl; 95% CI −23 to −5) in the premeal group relative to placebo. A significant decrease relative to placebo was present for mean 2-h postprandial insulin concentrations (−48 pmol/l; −79 to −17) with premeal GSK-1614235 dosing. This effect was less pronounced over 4 h. Similar to the effects seen for insulin, a significant decrease in C-peptide concentrations (−260 pmol/l; −435 to −85) was present. No effects were observed with either insulin or C-peptide with postmeal dosing.

The 3-OMG tracer was ingested with the breakfast meal to provide a more complete measure of plasma glucose absorption. Dosing with an SGLT1 inhibitor before breakfast resulted in a marked delay and decline in absorption of the tracer with an AUCl0−10 h of 231 μg·h−1·ml−1 (152–309) relative to 446 (381–511) and 411 (352 to 471) μg·h−1·ml−1, for placebo and postmeal dosing, respectively (Fig. 4). Any trend toward an effect with postmeal dosing was only noted beyond 1 h after breakfast. The 24-h recovery of the 3-g glucose tracer load in urine was 1.20 g with premeal dosing relative to 2.17 and 1.89 g for placebo and postmeal dosing, respectively. The range of urine recovery among individuals receiving 20 mg of GSK-1614235 before breakfast was quite broad, 0.11–2.59 g.

Figure 5 depicts mean concentrations of GIP, total GLP-1, and PYY for 4 h after breakfast. Postprandial immunoreactive GIP concentrations indicative of intestinal K cell secretion were reduced with premeal dosing for both 2 and 4 h following breakfast. Postmeal dosing also showed a suppression of GIP concentrations for the 4-h window. Opposite effects were observed for the products of intestinal L cell products, total GLP-1, and PYY. Total GLP-1 concentrations were significantly increased with both premeal and postmeal dosing, whereas a trend for increased PYY was only noted for the premeal dosing. Active GLP-1 data were not presented, since approximately two-thirds of samples fell below the lower limit quantification for the assay (2 pmol/l). Glucagon results (data not shown) were unchanged between the placebo and both GSK-1614235 dosing regimens.

Linear regression analysis of individual PD parameters vs. 3-OMG absorption indicated a linear relationship between PD response and SGLT1 inhibition for insulin ($P$ value for the slope = 0.0165), C-peptide ($P$ = 0.0074), and GIP ($P$ = 0.0107) with premeal dosing among the 12 subjects. Greater inhibition of glucose absorption was associated with larger decreases in postprandial weighted mean AUCl0−2 h for each of these three parameters.

**Pharmacokinetics.** The effect of timing of drug dosing relative to meal on pharmacokinetics of GSK-1614235 was evaluated. Single doses of GSK-1614235 administered premeal were rapidly absorbed with a median $t_{\text{max}} < 1$ h. Plasma concentrations indicated very low systemic exposure of the active parent molecule with rapid clearance. Postmeal dosing delayed absorption, and plasma GSK-1614235 exposures were approximately one-half of those following premeal dosing. Large intersubject variability was observed in GSK-1614235 PK parameters, with a coefficient of variation of ~200%. Plasma concentrations of two metabolites lacking SGLT1 activity were measured, and plasma exposures of both metabolites following postmeal GSK-1614235 dosing were similar to

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**Fig. 4.** 3-O-Methyl-α-D-glucopyranose (3-OMG) recovery in healthy human subjects. Plasma concentrations (A) after oral administration of 3 g of 3-OMG tracer. Cumulative recovery of 3-OMG in urine (B) after oral administration of tracer. Individual plasma recovery curves for subjects after administration of placebo (C) or 20 mg GSK-1614235 premeal (D). Premeal indicates 20 mg of GSK-1614235 administered immediately before breakfast, and postmeal indicates administration 30 min after the start of the meal. HRS, hours. Values are means ± SE.

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those following premeal dosing. Exposure to the metabolites was significantly greater than the active parent molecule (at least 25-fold for GSK-2313533 and 500-fold for GSK-2313537).

DISCUSSION

This is the first set of nonclinical and clinical studies that has determined the effects of selective SGLT1 inhibitors in animal models and confirmed their translation to normal human physiology. Both nonclinical and clinical studies included administration of an inert glucose analog, 3-OMG, to enhance the mechanistic understanding of the physiological response to SGLT1 inhibition. In vivo pharmacology studies demonstrated the impact of potent, selective SGLT1 inhibition on glucose tracer absorption in rats and showed that altered glucose absorption profoundly changed postprandial insulin and GIP responses. The first reported clinical administration of a selective SGLT1 inhibitor confirmed translation of nonclinical pharmacology results to the clinical setting when single doses of GSK-1614235 were administered to healthy subjects immediately before and 30 min after meals.

The clinical trial explored the link between SGLT1 inhibition, measured as delayed systemic appearance of the glucose tracer, and postprandial physiological responses. It also evaluated flexibility around the timing of GSK-1614235 dosing relative to meals. The glucose analog 3-OMG was administered with the morning meal. The 3-OMG tracer can be transported by the full array of glucose transporters, but it is not metabolized in rats and humans (5, 6, 34). Because the inhibitors demonstrated no effect on gastric emptying (data not shown), the appearance of 3-OMG in plasma and recovery in the urine reflected glucose absorption or malabsorption, respectively, and provided a direct measure of GSK-1614235 inhibition of glucose transport. In untreated human subjects, 72% (69–76%) of 3-OMG was recovered in urine. This value was consistent with values reported in the seminal validation study conducted by Fordtran et al. (13), who measured 82% (95% CI 63–100%) 24-h urine recovery in healthy individuals administered 20 g of 3-OMG solution in the absence of an accompanying meal. The same study also revealed that concomitant administration of glucose reduced the rate of absorption of the 3-OMG (13), but 24-h urine recovery was not measured with concomitant glucose or a standardized meal.

Administration of GSK-1614235 before meals reduced mean 3-OMG plasma AUC by 50% relative to placebo, with broad interindividual variability, whereas mean urine 3-OMG recovery was reduced 45%. These data confirm the preeminent role of SGLT1 in transporting glucose across the intestinal brush border following the ingestion of a meal. A considerably smaller impact on glucose absorption was observed when GSK-1614235 was administered 30 min after the meal, when glucose absorption had already reached its peak. Thus, timing of the SGLT1 inhibitor dose relative to meals would be expected to be an important determinant of the resultant clinical efficacy of GSK-1614235.

Plasma and urine tracer glucose dynamics measured for rodents in the presence and absence of SGLT1 inhibition translated quite well to the clinical setting. Total recovery of the administered tracer dose was 77% (69–85%) in plasma, urine, and feces of rats over the 24-h interval. SGLT1 inhibition decreased urine tracer recovery, increased fecal recovery from 4 to 29%, and increased the “unrecovered” fraction from 23 (15–31%) to 43% (32–55%). There were several potential explanations for reduced total recovery. For example, 3-OMG can be fermented by lower intestinal bacteria to form short-chain fatty acids and release the 3H in a form that was not completely measured. Alternatively, 3-OMG remained in total body water to be eliminated in urine or in the lumen of the intestine to be absorbed from the lower gut at a later time and eliminated in uncollected urine or feces (supported by plasma levels of 3-OMG measured at 24 h). More prolonged collection intervals for urine and feces would be expected to increase 3-OMG recovery. Because more than one-half of the labeled glucose was recovered from feces in the KGA-2727 group and the measurement of fecal concentration required multiple steps of extraction, the final calculated value could possibly be underestimated.
Given that postprandial glucose absorption was concordant between clinical and nonclinical studies, it is important to verify that metabolic and enteroendocrine responses were also concordant. Surprisingly, weighted mean (0-2 and 0-4 h) glucose concentrations in healthy human subjects were not decreased by GSK-1614235. The acute postprandial peak glucose concentration was attenuated, but glucose concentrations remained slightly elevated at 90 and 120 min with premeal GSK-1614235 dosing. The significant decrease in insulin and C-peptide concentrations with premeal GSK-1614235 dosing could explain the altered profile of postprandial glucose concentrations. With SGLT1 inhibition, decreased glucose absorption diminished the acute glucose stimulus to the β-cell, resulting in reduced acute insulin release and a delayed fall from peak glucose concentrations. Linear regression analysis of individual responses indicated that greater inhibition of glucose absorption correlated with larger decreases in 2-h postprandial insulin, C-peptide, and GIP responses.

A close association was also noted between the magnitude and timing of plasma 3-OMG concentrations and plasma GIP profiles, which is consistent with reports that secretion of GIP from K cells occurs in response to glucose in the lumen of humans and rodents (11, 14, 15, 17, 18). These clinical data indicate either that SGLT1 is the luminal glucose sensor in human K cells or that SGLT1 transport of glucose contributes to glucose sensing via a more indirect pathway (25, 27, 39). They are not consistent with reports that type 1 G protein-coupled receptor 2 and the type 1 G protein-coupled receptor sweet taste receptors at the apical membrane of enteroendocrine cells sense glucose concentrations in the intestinal lumen and regulate GIP release from K cells (16, 19, 30). Luminal glucose concentrations in the duodenum and jejunum would have remained higher when SGLT1 transport was blocked, yet GIP release was suppressed. On the other hand, more glucose substrates would have reached the distal portions of the ileum and colon (29), potentially augmenting the luminal glucose stimulus to enhance GLP-1 and PYY release from L cells in the distal gastrointestinal tract as reported for a dual SGLT1/SGLT2 inhibitor (41). Previous studies in rats have measured residual glucose contained in the stomach; upper, middle, and lower small intestine; and cecum at 1 and 3 h after starch administration (29). These data indicated that glucose was rapidly absorbed in the upper small intestine under normal circumstances, but a substantial fraction of ingested carbohydrate reached the lower small intestine and cecum after treatment with KGA-2727. The clinical trial did not include measures of active drug or 3-OMG concentrations in the intestinal lumen, so limited information is available regarding the competitive interaction of drug and SGLT1 substrates as they move along the gastrointestinal tract over time.

In conclusion, the specific SGLT1 inhibitors KGA-2727 and GSK-1614235 delayed and reduced glucose absorption, as measured by the glucose tracer 3-OMG. SGLT1 inhibition reduced peak postprandial glucose concentrations and diminished the requirement for acute insulin release, two factors associated with worsening of type 2 diabetes and its complications. Furthermore, changes in glucose absorption and the distribution of glucose substrates in the intestinal lumen significantly reduced secretion of GIP from K cells primarily distributed in the duodenum and enhanced GLP-1 and PYY release from L cells concentrated in the ileum and colon. The hormonal response to SGLT1 inhibition was generally consistent between rats and healthy human subjects and would be expected to regulate insulin secretion, appetite, and hepatic glucose production in a manner that would augment efficacy in hyperglycemic individuals.

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DISCLOSURES

The preclinical and clinical trials were sponsored by GlaxoSmithKline, plc. RLD, SLB, SMA, JAW, AW, LC, YL and CDS were employees of GlaxoSmithKline, FLG and the staff at Pennington Biomedical Research Center were contracted to perform the clinical trial.

AUTHOR CONTRIBUTIONS


REFERENCES

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