Synergistic Effects of a GPR119 Agonist with Metformin on Weight Loss in Diet-Induced Obese Mice

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ABSTRACT

G protein–coupled receptor 119 (GPR119) is a G protein–coupled receptor expressed predominantly in pancreatic β-cells and gastrointestinal enteroendocrine cells. Metformin is a first-line treatment of type 2 diabetes, with minimal weight loss in humans. In this study, we investigated the effects of GSK2041706 [2-[(1S)-1-(1-3-(1-methylethyl)-1,2,4-oxadiazol-5-yl)-4-piperidinyl]ethyl]oxy)-5-[(4-methylsulfonyl)phenyl]pyrazine], a GPR119 agonist, and metformin as monotherapy or in combination on body weight in a diet-induced obese (DIO) mouse model. Relative to vehicle controls, 14-day treatment with GSK2041706 (30 mg/kg b.i.d.) or metformin at 30 and 100 mg/kg b.i.d. alone caused a 7.4%, 3.5%, and 4.4% (all P < 0.05) weight loss, respectively. The combination of GSK2041706 with metformin at 30 or 100 mg/kg resulted in a 9.5% and 16.7% weight loss, respectively. The combination of GSK2041706 and metformin at 100 mg/kg caused a significantly greater weight loss than the projected additive weight loss of 11.8%. This body weight effect was predominantly due to a loss of fat. Cumulative food intake was reduced by 17.1% with GSK2041706 alone and 6.6% and 8.7% with metformin at 30 and 100 mg/kg, respectively. The combination of GSK2041706 with metformin caused greater reductions in cumulative food intake (22.2% at 30 mg/kg and 37.5% at 100 mg/kg) and higher fed plasma glucagon-like peptide 1 and peptide tyrosine tyrosine levels compared with their monotherapy groups. In addition, we characterized the effect of GSK2041706 and metformin as monotherapy or in combination on neuronal activation in the appetite regulating centers in fasted DIO mice. In conclusion, our data demonstrate the beneficial effects of combining a GPR119 agonist with metformin in the regulation of body weight in DIO mice.

Introduction

Obesity is a global epidemic and risk factor for developing type 2 diabetes mellitus (T2DM) (Stein and Colditz, 2004). Many patients with T2DM are obese (Eckel et al., 2011). Prevention or treatment of obesity will almost certainly benefit the incidence and care of T2DM. Identifying innovative new therapies, such as combination treatments that could improve glucose metabolism and reduce body weight, will provide benefits to patients with both obesity and type 2 diabetes.

The G protein–coupled receptor (GPCR) 119 (GPR119) has recently attracted attention because of preclinical and clinical evidence that its modulation may produce favorable effects on glucose homoeostasis, food intake and body weight gain, providing benefits to patients with both obesity and type 2 diabetes. GPR119 is a class A (rhodopsin-like) GPCR, with no close primary sequence relative in the human genome (Fredriksson et al., 2003), and its sequence is highly conserved in a human, mouse, and rat (Bonini et al., 2001).

GPR119 is expressed in human pancreatic α- and β-cells and the gastrointestinal tract (Bonini et al., 2001, 2002, Chu et al., 2008; Odori et al., 2013). In rodents, GPR119 mRNA was also detected primarily in the colon and small intestine, pancreas, and many areas of the rat and mouse brain (Bonini et al., 2001, 2002; Odori et al., 2013). The fatty acid amide oleoylthanolamide (EC50 = 3.2 or 4.4 μM) and the endovanilloid N-oleoyl dopamine (EC50 = 3.2 μM) and olvanil (EC50 = 7.8 μM) represent the most potent natural GPR119 agonists in vitro, although they are less potent and selective than the natural ligands identified for many other GPCRs (Overton et al., 2006; Chu et al., 2010).

Metformin is generally recommended as first-line treatment in patients with type 2 diabetes. It is an oral biguanide insulin-sensitizing agent that inhibits hepatic glucose production, enhances the effects of insulin on glucose uptake in skeletal muscles and adipocytes, and decreases intestinal absorption of glucose (Hundal et al., 1992; Klip et al., 1992; Wiernsperger and Bailey, 1999; Hardie, 2013). Metformin is also known to induce weight stabilization or small weight losses in diabetic

ABBREVIATIONS: AP, area postrema; DIO, diet-induced obese; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; GPCR, G protein–coupled receptor; GPR119, G protein–coupled receptor 119; GSK2041706, 2-[(1S)-1-(1-3-(1-methylethyl)-1,2,4-oxadiazol-5-yl)-4-piperidinyl]ethyl]oxy)-5-[(4-methylsulfonyl)phenyl]pyrazine; LH, lateral hypothalamus; NTS, nucleus tractus solitarius; PBS, phosphate-buffered saline; PNN, paraventricular nucleus; PYY, peptide tyrosine tyrosine; QMR, quantitative magnetic resonance; T2DM, type 2 diabetes mellitus; VMH, ventromedial nucleus.
and nondiabetic adults (UKPDS Group, 1998; Gueck et al., 2001; Kay et al., 2001; Seifarth et al., 2013). In addition, metformin enhances leptin sensitivity in high-fat–fed obese rats (Kim et al., 2006) and induces weight loss (metformin at 30 mg/kg caused a ~6% weight loss relative to vehicle by day 7 of treatment) in diet-induced obese (DIO) mice (Kim et al., 2013a).

When metformin and lifestyle changes are not enough to control a patient’s blood glucose levels, other medications are added as combination treatments to achieve better therapeutic efficacy. In addition, for type 2 diabetic patients with other comorbidities (e.g., obesity and dyslipidemia), metformin has been used in combination with other agents.

The GPR119 agonists have been proposed as a potential new approach for improving glucose metabolism in diabetic patients (Goodman et al., 2011). GSK1292263, a clinical GPR119 agonist, has been used in combination with other agents. Metformin is an effective oral medication for type 2 diabetes and is often used as monotherapy or in combination with other agents. However, in some cases, metformin may not be sufficient to control glucose levels, and additional medications are needed. GPR119 agonists, such as GSK1292263, could be used as an alternative or as a combination therapy to improve glucose metabolism and achieve better therapeutic control.

Materials and Methods

All studies were conducted after being reviewed by the GlaxoSmithKline Institutional Animal Care and Use Committee and in accordance with the GlaxoSmithKline policy on the care, welfare, and treatment of laboratory animals, Animal Welfare Act (U.S. Department of Agriculture), and Guide for Care and Use of Laboratory Animals.

Male DIO C57BL/6 mice (Taconic, Germantown, NY) were obtained at 20 weeks of age. The DIO mice were group housed and fed a high fat diet (60% fat by kilocalories) (D12492; Research Diets, New Brunswick, NJ) by the vendor from the time of weaning. For the purpose of accurate measurement of food intake, upon receipt, all mice were single housed and switched to a 45% high fat diet (D12451; Research Diets). Animals kept all the high fat–induced phenotypes after switching to a 45% high-fat diet. All mice were maintained at 72°F and 50% relative humidity with a 12-hour light/dark cycle from 5:00 AM to 5:00 PM and had free access to food and water. The DIO C57BL/6 mice were habituated in house for at least 2 weeks before the start of the study. The DIO mice were orally dosed with vehicle (0.5% hydroxypropyl methylcellulose/0.1% Tween 80, 10 ml/kg) at 9:00 AM and 4:00 PM (b.i.d.) for 10–15 days to acclimate them to handling and dosing stress before starting with drug treatment. The following studies were conducted in DIO mice:

1. GSK2041706 dose-range efficacy study: In this study, DIO mice were randomized according to their baseline body fat mass into four treatment groups with a similar mean body fat mass. Body composition was measured with quantitative magnetic resonance (QMR) (Echo Medical Systems, Houston, TX) on the last day of vehicle treatment. Then, animals were orally dosed twice a day (b.i.d.) with GSK2041706 at 0, 3, 10, and 30 mg/kg at 9:00 AM and 4:00 PM for 7 days. Body weight and food intake were measured.

2. Efficacy of GSK2041706 and metformin combination: after acclimation for handling and dosing, 42 DIO mice were randomized according to their body fat mass (measured by Echo QMR) into six groups (eight mice per group) to receive one of the following treatments: i) vehicle, ii) GSK2041706 (30 mg/kg), iii) metformin (30 mg/kg), iv) metformin (100 mg/kg), v) GSK2041706 (30 mg/kg) + metformin (30 mg/kg), or vi) GSK2041706 (30 mg/kg) + metformin (100 mg/kg). All treatments were oral dosing (b.i.d.) at 9:00 AM and 4:00 PM for 14 days. On day 13, body composition was measured using Echo QMR. On day 14, 1 hour post–oral dosing in the morning, blood was collected via cardiac puncture under isoflurane anesthesia, and plasma and serum were separated for further analysis (see “Blood Sample Collection and Analysis”). A sample (50 µl) of whole blood was added to a tube containing 50 µl of 0.5% EDTA/water (Shelton Scientific, Inc., Shelton, CT) for compound concentration measurement.

3. Acute effects of GSK2041706 on plasma peptide hormones: DIO mice were randomized according to their body weights into six groups (eight mice per group), with close average body weights (nonfasted). Mice were orally dosed at 9:00 AM either with vehicle (10 ml/kg, three groups) or GSK2041706 (30 mg/kg, three groups). Blood samples were collected by cardiac stick under isoflurane anesthesia at three time points (9:15, 9:30, and 10:00 AM) from a group treated with GSK2041706 and their respective vehicle controls.

4. c-fos immunohistochemistry studies: twenty-four DIO mice, which were handled and previously dosed with vehicle, were fasted overnight (22 hours) and randomized according to their body weights into six groups to receive one of the following treatments: vehicle, GSK2041706 (30 mg/kg), metformin (30 mg/kg), metformin (100 mg/kg), GSK2041706 (30 mg/kg) + metformin (30 mg/kg), and GSK2041706 (30 mg/kg) + metformin (100 mg/kg). Two and a half hours following oral dosing (i.e., at 1:00 PM), each mouse was anesthetized under isoflurane inhalation, and a blood sample was collected by cardiac stick for plasma hormone analysis before being transcardially perfused with 4% buffered paraformaldehyde. The whole brain was rapidly removed and immediately placed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) (pH 7) (Electronic Microscopy Sciences, Hatfield, PA). Twenty-four hours after fixation, the brains were immersed in 30% sucrose (Sigma-Aldrich, St. Louis, MO).

Brain Preparation for c-fos Analysis. After brain fixation for 24 hours, brains were serial sectioned at 30 µm and immunohistochemistry was performed as follows: the sections were blocked with normal goat serum (150 µl/10 ml PBS; Vector Laboratories, Burlingame, CA) for 30 minutes and then incubated overnight with a c-fos rabbit antibody (sc-52; Santa Cruz Biotechnologies, Dallas, TX) at 1:500 in PBS/0.3% Triton X-100 (Sigma-Aldrich). The sections were then incubated with biotinylated goat anti-rabbit (Vector Laboratories) at 5 µl/ml in PBS/0.3% Triton X-100 for 30 minutes followed by Vector ABC (100 µl of A, 100 µl of B/10 ml PBS; Vector Laboratories) for 30 minutes. Finally, the sections were incubated with DAB (1 drop/ml; Dako, Carpinteria, CA) for 2 minutes, which produces a brown precipitate in c-fos–positive nuclei. After cover slipping and drying, the slides were scanned with NanoZoomer (Hamamatsu, Hamamatsu, Japan) to produce digital images that were then analyzed with Tissue Studio (Definiens AG, Munich, Germany) to quantitate the number of c-fos–positive nuclei in the brainstem area postrema (AP), nucleus tractus solitarius (NTS), hypothalamic paraventricular nucleus (PVN), ventromedial nucleus (VMH), and lateral hypothalamus (LH).

Blood Sample Collection and Analysis. Blood samples from the above studies were collected via a cardiac stick under isoflurane anesthesia. EDTA tubes (Terumo Medical Corporation, Elkton, MD) containing 50 µM dipyridyl peptide IV inhibitor (Millipore, Cat. No. 302052) were used to collect blood samples. Blood samples were collected from a central cannula under isoflurane inhalation at three time points (9:15, 9:30, and 10:00 AM) from a group treated with GSK2041706 and their respective vehicle controls.
St. Charles, MO) and a protease inhibitor cocktail (Sigma-Aldrich) were used to prepare plasma for hormone analysis. Plasma hormone levels were analyzed using the MilliPlex MAP mouse gut hormone panel for insulin, amylin, leptin, ghrelin, glucose-dependent insulintropic polypeptide (GIP), and PYY. Meso Scale Discovery assays were used for measurement of active and total GLP-1 (Meso Scale Discovery, Gaithersburg, MD). Blood serum was prepared by using T-MG tubes (Terumo Medical Corporation). Clinical chemistries were determined by analyzing serum samples using a Beckman Coulter Olympus AU640 clinical chemistry analyzer (Beckman Coulter Inc., Brea, CA).

**Statistical Analysis.** Data are presented as mean ± S.E.M., with n indicating the number of animals per group. The data were analyzed using JMP (9.0.1; SAS Institute, Cary, NC) software. A one-way analysis of variance with post hoc Student’s t test was applied to compare treatments versus vehicle controls. Body weight data were analyzed through day 7 or 14 of treatment using a repeated measure analysis of covariance, with a compound symmetric covariance structure. Treatment means were compared using a post hoc t test. P values < 0.05 were considered to indicate a significant difference between treatment groups.

**Results**

**GSK2041706 Dose-Range Efficacy.** GSK2041706 caused a dose-dependent weight loss in DIO mice (Fig. 1A). Relative to changes in the vehicle group, administration of GSK2041706 at 30 mg/kg b.i.d. caused a 3.6 ± 0.6% weight loss on day 7 of treatment (P < 0.05 versus vehicle). Lower doses of GSK2041706 (3 and 10 mg/kg) failed to cause a significant weight loss (0.3 ± 0.7% and -1.1 ± 0.5% weight loss from vehicle, respectively; P > 0.05). Cumulative food intake over the seven days of treatment was significantly reduced by 16.5% with GSK2041706 at 30 mg/kg (14.4 ± 0.8 g; P < 0.05) relative to vehicle (17.2 ± 0.4 g).

There was only a 4.9% reduction in cumulative food intake at the 10 mg/kg dose (16.3 ± 0.6 g), but no obvious effect at the 3 mg/kg dose (17.6 ± 0.6 g) (Fig. 1B). The dose of 30 mg/kg was selected to be used for the evaluation of GSK2041706 in other studies.

**Efficacy of GSK2041706 and Metformin Combination.** In a chronic 14-day study, treatment with GSK2041706 (30 mg/kg) alone caused a 7.4 ± 1.4% weight loss (from baseline and corrected to vehicle control) by day 14 (P < 0.05) in DIO mice (Fig. 2A). Metformin treatment at 30 and 100 mg/kg caused a 3.5 ± 0.9% and 4.4 ± 1.1% weight loss, respectively (P < 0.05). The combination of GSK2041706 + metformin at 30 and 100 mg/kg caused a 9.5 ± 0.7% and 16.7 ± 1.7% (P < 0.05) weight loss, respectively, compared with vehicle. The combination of GSK2041706 + metformin at 100 mg/kg, but not at 30 mg/kg, caused a significantly greater (5% more) weight loss than the predicted additivity (11.7%) of the weight losses caused by GSK2041706 and metformin (100 mg/kg) monotherapy (Fig. 2A). Cumulative food intake (Fig. 2B) was significantly reduced in groups treated with GSK2041706 (17.1%; P < 0.05) and metformin at 30 mg/kg (6.6%; P = 0.468) and 100 mg/kg (8.7%; P < 0.05) monotherapy. The combination of GSK2041706 + metformin at 30 and 100 mg/kg significantly reduced cumulative food intake by 22.2% and 37.6%, respectively (P < 0.05). The reduction in cumulative food intake caused by the combination of GSK2041706 + metformin at 100 mg/kg was significantly greater than the predicted additivity (25.8%; P < 0.05) of these two agents as monotherapy (Fig. 2B). The inhibition of the cumulative food intake mirrors the weight loss seen in DIO mice on day 14 of treatment, suggesting that the inhibition of food intake is part of the mechanism of action of GSK2041706.

![Fig. 1. Inhibition of food intake and induction of body weight loss in DIO mice by GSK2041706. Vehicle or GSK2041706 at the indicated doses was orally administered b.i.d. (at 9:00 AM and 4:00 PM) to DIO C57BL/6 mice for seven days. Cumulative food intake over the seven days of treatment (B) and body weight (A) expressed as a percentage change from baseline body weight and adjusted to vehicle controls. Average body weight on day 0: 42.7 ± 0.5 g (n = 31). *P < 0.05 versus vehicle.](image-url)
of the mechanism by which those agents caused weight loss while given as monotherapy or in combination. Comparison of the weight loss with the cumulative reduction in food intake by converting the amount of weight loss (using a conversion of 9 kcal/g) and cumulative food intake (4.73 kcal/g) to kilocalories revealed that the weight loss mirrored the reduction in cumulative food intake (Fig. 3, A and B).

Evaluation of the change in body fat and nonfat mass on day 13 of treatment from the pretreatment baseline revealed that there was a significant reduction in body fat mass in all of the treatment groups, except in the group treated with metformin at 30 mg/kg alone. Consistent with the body weight loss, the combination of GSK2041706 and metformin (100 mg/kg) caused a significantly greater fat mass loss (−6.25 ± 0.47 g versus 1.11 ± 0.38 g for vehicle; P < 0.05) than the sum of GSK2041706 (−1.37 ± 0.56 g) and metformin (100 mg/kg) (−0.69 ± 0.24 g) as monotherapy. The combination of GSK2041706 + metformin at 30 mg/kg (0.14 ± 0.29 g) caused a significant fat mass loss of 2.93 ± 0.41 g relative to vehicle (P < 0.05). GSK2041706 dosed alone at 30 mg/kg was the only group that showed a little but statistically significant reduction of nonfat mass compared with the vehicle group (−1.12 g versus vehicle −0.69 g; P < 0.05) (Fig. 4).

Plasma peptide hormones and serum chemistries measured on day 14 of treatment, 1 hour post–morning dosing in fed DIO mice are shown in Tables 1 and 2. Plasma insulin concentrations were significantly reduced in all treatment groups (P < 0.05), except in the group treated with GSK2041706 alone. Treatment with metformin reduced plasma insulin levels by 35.6% and 54.8% at 30 and 100 mg/kg relative to the vehicle group, respectively (P < 0.05). The combination of GSK2041706 with metformin at 30 and 100 mg/kg reduced plasma insulin levels by 78% and 75.4%, respectively. The plasma amylin level was significantly reduced (52.3%; P < 0.05) in the combination group of GSK2041706 and metformin at 100 mg/kg. Plasma leptin levels were lower in all treatment groups relative to the vehicle group and reached significance only in the GSK2041706 + metformin 100 mg/kg group (22.3% versus vehicle; P < 0.05). Plasma GIP concentrations were significantly reduced by metformin at 100 mg/kg (57.2%; P < 0.05) alone and in combination with GSK2041706 (69.1%; P < 0.05). Plasma PYY levels trended up with GSK2041706 (28.3%) and trended down with metformin alone at 30 and 100 mg/kg by 14% and 25%, respectively. However, relative to vehicle, the combination of GSK2041706 + metformin at 30 and 100 mg/kg significantly increased plasma PYY levels by 38.5% and 146.3%, respectively (P < 0.05; Table 1). Plasma active GLP-1 levels were significantly reduced in the GSK2041706 group treated alone (39.7%; P < 0.05), whereas they were significantly increased by 68.5% and 120.2% with metformin alone at 30 and 100 mg/kg, respectively. The combination of GSK2041706 + metformin at 30 and 100 mg/kg significantly increased plasma active GLP-1 levels by 105.1% and 251.3%, respectively (P < 0.05). Relative to the vehicle group, plasma total GLP-1 was increased by 23% (P = 0.249) and 26% (P < 0.05) with metformin at 30 and 100 mg/kg, respectively. The combination of GSK2041706 + metformin
at 30 and 100 mg/kg increased the total GLP-1 level by 26.2% and 209.9%, respectively ($P < 0.05$).

A summary of serum chemistries is shown in Table 2. Relative to the vehicle group, treatment with GSK2041706 alone reduced total cholesterol (19.3%; $P < 0.05$) and triglyceride (11.5%; $P = 0.149$) levels. Metformin alone caused no detectable change in serum total cholesterol or triglyceride levels. However, the combination of GSK2041706 + metformin significantly decreased the levels of total cholesterol (19% and 30.8% at 30 and 100 mg/kg, respectively) and triglycerides (25.8% and 43.6% at 30 and 100 mg/kg, respectively). Changes in serum cholesterol–high-density lipoprotein levels mirrored changes in total cholesterol levels. Treatment with metformin alone significantly increased non–esterified fatty acid and decreased serum glycerol levels. The combination of GSK2041706 + metformin at 30 and 100 mg/kg significantly reduced glycerol levels by 29.8% and 35.1%, respectively ($P < 0.05$). Treatment with GSK2041706 alone or in combination with metformin at 100 mg/kg significantly increased serum $\beta$-hydroxybutyric acid, a marker of fatty acid oxidation, by 55.5% and 53.8%, respectively ($P < 0.05$). The combination of GSK2041706 + metformin (30 and 100 mg/kg) maintained or increased more
levels of serum β-hydroxybutyric acid (41.3% and 148.1%, respectively; $P < 0.05$).

Measurement of whole blood GSK2041706 and metformin concentrations 1 hour post–last oral dose on day 14 revealed that GSK2041706 (9235 ± 1320 ng/ml) and metformin at 30 (4310 ± 1796 ng/ml) and 100 mg/kg (8232 ± 3240 ng/ml) (data are mean ± S.D.) are well absorbed, and the combination of metformin at 30 mg/kg (3624 ± 1223 ng/ml) or 100 mg/kg (8760 ± 3832 ng/ml) with GSK2041706 (9329 ± 382 and 8093 ± 999 ng/ml, respectively) has no obvious effect on the blood concentrations achieved.

**Acute Effects of GSK2041706 on Plasma Peptide Hormones.** Because the effects of GSK2041706 on peptide hormones in chronic dosing were different from previous reports with GPR119 agonists (e.g., insulin), we tested GSK2041706 acutely in nonfasted DIO mice. Relative to vehicle controls, GSK2041706 significantly increased plasma insulin concentration by 4.6- and 2-fold at 30 and 60 minutes, respectively ($P < 0.05$; Table 3). Plasma amylin concentration was significantly increased by 2.1-fold at 30 minutes ($P < 0.05$). Plasma GIP concentration was significantly elevated at 15 minutes (1.9-fold), reached a maximum of 2.8-fold at 30 minutes, and then reduced to 2.1-fold by 60 minutes ($P < 0.05$). Plasma PYY levels were significantly increased at 15 minutes (1.9-fold) and 30 minutes (2.3-fold) ($P < 0.05$). Plasma active GLP-1 concentration reached a maximum of 3.4-fold at 15 minutes ($P < 0.05$) and then trended lower to 2-fold at 30 minutes ($P < 0.05$). Total GLP-1 levels significantly increased by 2.8-fold at 15 minutes, reached

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin</th>
<th>Amylin</th>
<th>Leptin</th>
<th>GIP</th>
<th>PYY</th>
<th>aGLP-1</th>
<th>tGLP-1</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>2841 ± 277</td>
<td>312.7 ± 35.1 (n = 7)</td>
<td>22,757 ± 2399</td>
<td>269.4 ± 56.6</td>
<td>168.4 ± 10.8 (n = 7)</td>
<td>2.4 ± 0.3</td>
<td>21.3 ± 1.7</td>
</tr>
<tr>
<td>GSK2041706 (30 mg/kg)</td>
<td>2094 ± 320</td>
<td>264.4 ± 21</td>
<td>20,108 ± 1510</td>
<td>275.3 ± 42.2</td>
<td>216 ± 14.5</td>
<td>1.4 ± 0.3*</td>
<td>19.4 ± 1.7</td>
</tr>
<tr>
<td>Metformin (30 mg/kg)</td>
<td>1830 ± 651*</td>
<td>227.4 ± 42</td>
<td>21,129 ± 3161</td>
<td>212.5 ± 35</td>
<td>144.7 ± 27.9</td>
<td>4 ± 0.3*</td>
<td>26.2 ± 1.3*</td>
</tr>
<tr>
<td>Metformin (100 mg/kg)</td>
<td>1283 ± 196*</td>
<td>187.3 ± 16.3</td>
<td>21,072 ± 2067</td>
<td>115.3 ± 13.8*</td>
<td>126.2 ± 20.1 (n = 7)</td>
<td>5.2 ± 0.7*</td>
<td>26.8 ± 2.8</td>
</tr>
<tr>
<td>GSK2041706 + metformin (30 mg/kg)</td>
<td>1208 ± 140*</td>
<td>201.8 ± 18.5</td>
<td>19,585 ± 1780</td>
<td>160.3 ± 27.1</td>
<td>251.4 ± 29.4*</td>
<td>3.9 ± 0.4*</td>
<td>26.4 ± 1.4*</td>
</tr>
<tr>
<td>GSK2041706 + metformin (100 mg/kg)</td>
<td>699 ± 138*</td>
<td>131.5 ± 18.4*</td>
<td>12,750 ± 1457* (n = 7)</td>
<td>83.2 ± 13.2*</td>
<td>414.7 ± 43.1*</td>
<td>8.3 ± 0.6*</td>
<td>65.9 ± 6.5*</td>
</tr>
</tbody>
</table>

*aGLP-1, active GLP-1; tGLP-1, total GLP-1. $^*P < 0.05$ versus vehicle.
a maximum of 3-fold at 30 minutes, and then trended lower to 2.5-fold at 60 minutes (P < 0.05) (Table 3).

c-fos Immunohistochemistry Studies. Relative to vehicle controls, little to no change in c-fos immunoreactivity was seen with GSK2041706 or with the lower dose of metformin in brainstem AP, NTS, and hypothalamic PVN, VMH, and LH, with the exception of the AP, where a 7- to 8-fold increase was seen with the lower dose (30 mg/kg) of metformin (Table 4). With the higher (100 mg/kg) dose of metformin, a 5.4-fold increase in c-fos immunoreactivity was observed in the AP, and a 4.8- and 5.3-fold increase was observed in the NTS and PVN. GSK2041706 in combination with metformin at 30 mg/kg produced a 4.5-fold increase in AP and a 2.1-fold increase in the VMH area (P < 0.05 versus vehicle), whereas the combination of GSK2041706 with the higher metformin dose (100 mg/kg) tended to reduce the increased c-fos immunoreactivity induced by metformin alone, which was seen in AP, PVN, and LH, to vehicle levels. The areas unaffected were NTS and VMH, where c-fos levels for the combination remained the same as the high dose of metformin alone.

Plasma hormones collected 2.5 hours postdosing of GSK2041706 or metformin alone or in combination in overnight fasted DIO mice are summarized in Table 5. Relative to vehicle controls, GSK2041706 significantly increased plasma total GLP-1 by 2-fold, whereas metformin at 30 and 100 mg/kg significantly increased plasma active (2.4- and 3.5-fold, respectively) and total GLP-1 (1.7- and 2.5-fold, respectively). The combination of GSK2041706 and metformin at 30 mg/kg increased plasma active GLP-1 by 2.3-fold (P < 0.05) and total GLP-1 by 2-fold (P < 0.05). The combination of GSK2041706 with metformin at 100 mg/kg caused a 7.6-fold increase in plasma active and total GLP-1 (P < 0.05) and increased plasma PYY concentration by 3-fold. The combination of GSK2041706 with metformin at 30 and 100 mg/kg significantly increased plasma GIP levels by 2.2- and 2.5-fold, respectively (P < 0.05).

Discussion

The aforementioned studies demonstrated that the combination of GSK2041706 and metformin elicited a synergistic weight loss after chronic treatment than either monotherapy. The weight loss was due predominately to fat loss driven by a reduction in cumulative food intake. Greater weight loss in combination treatment was associated with higher plasma GLP-1 and PYY levels compared with the monotherapies. In addition, GSK2041706 induced no neuronal activation as a monotherapy but attenuated the c-fos immunoreactivity induced by metformin in AP, NTS, and PVN brain areas when the two agents were administered in combination in DIO mice.

Previous studies have shown that chronic treatment with GPR119 agonists, such as PSN632408 or HD0471953, caused inhibition of cumulative food intake and weight gain in a DIO rat model or weight loss in db/db mice, respectively (Overton et al., 2006; Kim et al., 2013a,b). In overweight/obese patients with type 2 diabetes, treatment with PSN821 for 14 days increased PYY levels and induced a substantial reduction in energy intake (Goodman et al., 2011). Chronic treatment with metformin was also reported to cause a reduction in food intake.
intake and weight loss in DIO mice (Zhu et al., 2013) or inhibit food intake in DIO rats (Aubert et al., 2011). In type 2 diabetic patients, metformin was reported to cause small amounts of weight loss without a change in energy expenditure (Stumvoll et al., 1995). In agreement with those reports, treatment with GSK2041706 or metformin as a monotherapy reduced cumulative food intake and caused weight loss and fat mass loss in DIO mice. More important, synergistic weight loss was achieved with combination treatment. The improved weight loss efficacy was predominately due to greater loss of fat mass associated with a significant reduction in plasma leptin, greater reduction in food intake, and increased fatty acid oxidation, which was indicated by higher serum β-hydroxybutyric acid, in the combination group compared with the monotherapies. Since the cumulative reduction in caloric consumption was equivalent to the calculated calories from weight loss, we conclude that GSK2041706 and metformin as a monotherapy or in combination induced fat loss through the suppression of food consumption in DIO mice. This is consistent with a previous report by Overton et al. (2006) that the inhibition of weight gain in DIO rats chronically treated with PSN632408 was predominantly due to sustained suppression of food intake without significant changes in energy expenditure or taste aversion. It is also in agreement with the findings that the reduction in food intake, but not changes in energy expenditure, contributed to the fat mass and weight loss observed in DIO mice (Kim et al., 2013a) and type 2 diabetic patients (Stumvoll et al., 1995) treated with metformin.

Expression of GPR119 has also been reported in certain areas of the mouse brain, including AP, PVN, and LH (Overton et al., 2006). However, GSK2041706 alone has no effect on neuronal activation in brainstem AP and NTS and hypothalamic PVN, VMH, and LH in DIO mice. The induction of c-fos immunoreactivity by metformin at 100 mg/kg in AP, NTS, and PVN is consistent with a previous report showing that metformin (at 300 mg/kg) significantly increased c-fos immunoreactivity in the NTS of DIO mice (Kim et al., 2013a). The authors suggested that metformin-induced GLP-1 secretion from intestinal L cells could be a possible mechanism. GLP-1 and neuropeptide Y receptor 2 receptors are expressed in brainstem AP and NTS and hypothalamic PVN, which are areas known to receive and integrate peripheral signals to induce satiety and weight loss (Parker and Herzog, 1999; Young, 2012). Both PYY1-36 and PYY have been reported to induce c-fos in the hypothalamus in rodents (Turton et al., 1996; Batterham et al., 2002). Recently, we demonstrated that the combination of PYY and GLP-1 induced a synergistic weight loss in DIO mice (Paulik et al., 2011). Surprisingly, the increased c-fos immunoreactivity induced by metformin in AP, NTS, and PVN was attenuated by GSK2041706 when given in combination, despite higher plasma PYY and GLP-1 levels in the combination group compared with metformin monotherapy. Further studies are needed to confirm this observation and understand underlying mechanisms. A similar finding reported by Williams et al. (2006) demonstrated that the combination of leptin with exendin-4 synergistically reduced food intake and body weight in rats. They found that the c-fos immunoreactivity that was induced by exendin-4 in AP and NTS was attenuated by pretreatment with leptin. In the same study, treatment with leptin alone reduced food intake without inducing a change in c-fos immunoreactivity.

In conclusion, the present study is the first, to our knowledge, to report the synergistic weight loss effect of a GPR119 agonist with metformin in DIO mice. This preclinical finding may suggest potential beneficial effects of combining a GPR119 agonist with metformin in the treatment of obese diabetic patients.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AP</th>
<th>NTS</th>
<th>PVN</th>
<th>VMH</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>7.75 ± 2.59</td>
<td>23.75 ± 12.69</td>
<td>57.5 ± 14.82</td>
<td>69 ± 26.5</td>
<td>60.00 ± 20.45</td>
</tr>
<tr>
<td>GSK2041706 (30 mg/kg)</td>
<td>8.75 ± 4.13</td>
<td>5.00 ± 2.04</td>
<td>26.0 ± 3.72</td>
<td>93.5 ± 13.29</td>
<td>62.25 ± 12.66</td>
</tr>
<tr>
<td>Metformin (30 mg/kg)</td>
<td>61.25 ± 21.51</td>
<td>72.75 ± 41.88</td>
<td>84.25 ± 32.89</td>
<td>106.75 ± 10.45</td>
<td>77.25 ± 11.56</td>
</tr>
<tr>
<td>Metformin (100 mg/kg)</td>
<td>41.50 ± 6.98</td>
<td>14.27 ± 27.36</td>
<td>305.75 ± 42.03</td>
<td>133.50 ± 23.95</td>
<td>51.50 ± 17.00</td>
</tr>
<tr>
<td>GSK2041706 + metformin (30 mg/kg)</td>
<td>42.25 ± 5.34</td>
<td>3.50 ± 3.76</td>
<td>61.25 ± 15.91</td>
<td>145.25 ± 21.25</td>
<td>77.00 ± 14.59</td>
</tr>
<tr>
<td>GSK2041706 + metformin (100 mg/kg)</td>
<td>17.57 ± 6.25</td>
<td>107.75 ± 59.1</td>
<td>65.5 ± 16.81</td>
<td>112.00 ± 28.53</td>
<td>22.75 ± 3.35</td>
</tr>
</tbody>
</table>

*P < 0.05 versus vehicle.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin</th>
<th>Amylin</th>
<th>GIP</th>
<th>PYY</th>
<th>aGLP-1</th>
<th>tGLP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>2126 ± 301</td>
<td>173 ± 18.1</td>
<td>240 ± 29.3</td>
<td>190 ± 54</td>
<td>0.8 ± 1.0</td>
<td>15 ± 1.1</td>
</tr>
<tr>
<td>GSK2041706 (30 mg/kg)</td>
<td>2451 ± 301</td>
<td>157 ± 29.2</td>
<td>464 ± 108.6</td>
<td>152 ± 20.4</td>
<td>1.2 ± 0.3</td>
<td>20 ± 2.1</td>
</tr>
<tr>
<td>Metformin (30 mg/kg)</td>
<td>1800 ± 349</td>
<td>102 ± 26.2</td>
<td>162 ± 36.8</td>
<td>161 ± 27.8</td>
<td>1.9 ± 0.2*</td>
<td>26 ± 2.8*</td>
</tr>
<tr>
<td>Metformin (100 mg/kg)</td>
<td>1125 ± 449</td>
<td>105 ± 19.6*</td>
<td>227 ± 32.4</td>
<td>297 ± 67.6</td>
<td>2.8 ± 0.3*</td>
<td>37 ± 4.7*</td>
</tr>
<tr>
<td>GSK2041706 + metformin (30 mg/kg)</td>
<td>1998 ± 348</td>
<td>135 ± 44.8</td>
<td>525 ± 64.4*</td>
<td>143 ± 41.1</td>
<td>1.8 ± 0.5</td>
<td>29 ± 4.7*</td>
</tr>
<tr>
<td>GSK2041706 + metformin (100 mg/kg)</td>
<td>2584 ± 423</td>
<td>169 ± 43.1</td>
<td>602 ± 90.4*</td>
<td>597 ± 170.8*</td>
<td>6.1 ± 1.7*</td>
<td>114 ± 19.7*</td>
</tr>
</tbody>
</table>

aGLP-1, active GLP-1; tGLP-1, total GLP-1.
Authorship Contributions

Participated in research design: Al-Barazanji, Chen.
Conducted experiments: Al-Barazanji, McNulty, Benson.
Contributed new reagents or analytic tools: Binz, Generaux.
Performed data analysis: Al-Barazanji, Young, Benson.
Wrote or contributed to the writing of the manuscript: Al-Barazanji, Chen, Generaux, Yong, Benson.

References


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