Inhibition of apical sodium-dependent bile acid transporter as a novel treatment for diabetes

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Inhibition of apical sodium-dependent bile acid transporter (Asbt) is known as an elusive goal of pharmacological reduction of cardiovascular risk in type 2 diabetes mellitus. This article presents the results of a study investigating the effect of 264W94, a novel bile acid transporter inhibitor, on glycemic control in Zucker Diabetic Fatty (ZDF) rats. Oral administration of 264W94 for two weeks increased fecal bile acid concentrations and elevated non-fasting plasma total glucose. Treatment with 264W94 significantly decreased HbA1c and prevented the drop of insulin levels typical of ZDF rats in a dose-dependent manner. An oral glucose tolerance test revealed a significant reduction of blood glucose and LDL in T2DM patients. The implications of these findings for the development of new therapeutic strategies for type 2 diabetes mellitus are discussed.

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increasing free bile acids in the distal intestine might improve glucose metabolism, we evaluated the effects of 264W94 on glucose homeostasis in ZDF rats and explored potential underlying mechanisms.

RESEARCH DESIGN AND METHODS

Materials. GW4064 (23) and 264W94 (32) were synthesized by medicinal chemistry groups at GlaxoSmithKline.

Animal preparation. In the first study (study 1), male ZDF (ZDF/GmiCrl-ja/faj) were purchased from Charles River (Raleigh, NC) and housed under controlled conditions (12:12-h light-dark cycle, 24°C, and 50% relative humidity) with free access to rodent food (Purina 5008; Harlan Teklad, Indianapolis, IN). All rats arrived at 7 wk of age (±3 days). After a 1-wk acclimation period, rats were anesthetized with isoflurane (Abbott Laboratories), and tail vein blood samples were collected at 9 AM without fasting. Blood glucose levels were measured using a glucometer (Bayer, Leverkusen, Germany). To ensure balanced treatment groups, ZDF rats were assigned to six treatment groups based on baseline glucose: vehicle [0.5% hydroxypropyl methylcellulose (HPMC), 0.1% Tween 80] and five doses of 264W94 (0.001, 0.01, 0.1, 1, and 10 mg/kg). All treatments were given via oral gavage twice a day, and animals were followed for 2 wk with blood samples collected from tail vein at the end of each week at 9 AM without fasting. Three groups of the animals (vehicle and 0.1 and 10 mg/kg) were cannulated (jugular vein) at the beginning of the third week, and an oral glucose tolerance test (OGTT) was conducted at the end of the third week. Fecal samples were collected for 24 h during the second week of treatment.

In a separate study (study 2), 8-wk-old male ZDF rats were assigned to vehicle or 264W94 (1 mg/kg bid) groups based on baseline glucose and treated for 5 wk. A group of age-matched Zucker lean control rats was also included in the study. At the end of the study, intestinal (0.5- to 1-cm sections from the middle of duodenum, jejunum, ileum, ascending colon, and descending colon) and liver tissues were collected at 10 AM without fasting, and the mRNA expression of certain selected genes was determined using RT-qPCR.

In a third study (study 3), 8-wk-old male ZDF rats were assigned to four groups based on baseline glucose: vehicle, 264W94 (1 mg/kg bid), GW4064 (an Fxr agonist; 30 mg/kg bid) (23) and 264W94 + GW4064 (1 mg/kg and 30 mg/kg bid, respectively). Animals were treated for 2 days, and steady-state blood glucose was measured using glucometers at 9 AM without fasting.

Principles of laboratory animal care were followed. All procedures performed were in compliance with the Animal Welfare Act and US Department of Agriculture regulations and were approved by the GlaxoSmithKline Animal Care and Use Committee.

OGTT. Rats were fasted overnight (16 h) prior to OGTTs. Dextrose at 2 g/kg body wt was orally administered via gavage. Blood samples were collected via jugular vein cannulation immediately before and 5, 10, 20, 30, 60, 90, and 120 min after the administration of dextrose. Blood glucose levels were measured using glucometers (Bayer, Leverkusen, Germany), and plasma samples were collected for further analysis.

Measurement of clinical chemistry parameters. Plasma glucose, nonesterified fatty acids (NEFA), bile acids, and bile acids in fecal extraction were measured using the Olympus AU640 clinical chemistry analyzer (Beckman Coulter, Irving, TX). Glucose test reagents were manufactured by Beckman Coulter. NEFA reagents were manufactured by Wako and bile acids reagents by Trinity Biotech. Hb A1c was measured by the Primus Affinity Ultra2 HPLC System using Primus Affinity Assay reagents (Primus Diagnostics, Kansas City, MO). Insulin was assayed using the Meso Scale Discovery (MSD) Mouse/Rat Insulin assay kit. Total Glp-1 was assayed by the MSD Total Glp-1 assay kit. Both assays were analyzed on an MSD Sector Imager 6000 (Meso Scale Discovery, Gaithersburg, MD). Plasma proinsulin was determined by ELISA (ALPCO, Salem, NH).

RESULTS

Changes in fecal bile acid excretion and plasma bile acid concentrations. Oral administration of 264W94 dose-dependently increased bile acids in the feces. Fecal bile acid concentrations were elevated up to 6.5-fold with an ED50 of 0.15 mg/kg compared with vehicle-treated rats (Fig. 1A). Fecal NEFA also slightly increased in 264W94-treated rats (Fig. 1B).

In contrast, plasma bile acid concentrations were decreased dose-dependently in 264W94-treated rats (Fig. 1C).

Effects of 264W94 on glycemic control in ZDF rats. All groups of ZDF rats had comparable levels of plasma glucose, insulin, and blood Hb A1c prior to the start of treatment (Fig. 2, A, C, and E). The average baselines plasma glucose and Hb A1c in ZDF rats were 327 ± 13 mg/dl and 4.8 ± 0.04%, respectively. As illustrated in Fig. 2A, during the 2-wk study, the vehicle-treated rats showed a marked progressive hyperglycemia with plasma glucose and Hb A1c reaching 522 ± 28 mg/kg and 7.2 ± 0.2%, respectively. In contrast, plasma glucose in 264W94-treated rats was reduced dose-dependently compared with the vehicle-treated group. The efficacious doses of 264W94 (0.1 mg/kg and above) reduced plasma glucose concentrations below the baseline level (Fig. 2, A and B). Hb A1c measured at the end of 2-wk treatment showed a dose-dependent decrease compared with vehicle-treated group (Fig. 2, C and D). Baseline insulin (Fig. 2E) and proinsulin (data not shown) concentrations were similar across the treatment groups. The vehicle-treated rats showed a marked decrease in plasma insulin levels. Although the plasma concentration of proinsulin (data not shown) did not change in vehicle-treated animals, the proinsulin/insulin ratio increased (Fig. 2F), indicating β-cell dysfunction over the course of the study. Treatment of 264W94 prevented the decline of insulin dose-dependently without an increase in proinsulin levels.

To explore potential mechanisms of Asbt inhibition-induced glycemic control, steady-state nonfasting plasma total Glp-1 was measured at the beginning and on day 7 and day 14 of study. As shown in Fig. 2, G and H, treatment with 264W94...
resulted in an elevation of plasma total Glp-1 in a dose-dependent manner. Plasma total Glp-1 increased up to 50% at the doses of 1 and 10 mg/kg compared with the vehicle group. At 0.1 mg/kg, 264W94 significantly decreased blood glucose and Hb A1c in ZDF rats (Fig. 2, A and B) but had minimal effects on steady-state nonfasting plasma total Glp-1 (Fig. 2, G and H). To explore whether 264W94 at 0.1 mg/kg could improve postprandial dynamic Glp-1, an OGTT was performed at the end of study 1.

Responses of plasma glucose, insulin, and total glp-1 during OGTT. To further investigate dynamic mechanisms for Asbt inhibition-induced improvement of glucose homeostasis, we performed an OGTT in three treatment groups: vehicle and 0.1 and 10 mg/kg of 264W94 after 3 wk of treatment. Both 264W94-treated groups had significantly lower fasting blood glucose than the vehicle group (Fig. 3A). The 264W94-treated rats also showed a trend of reduced postprandial glucose excursion (Fig. 3, A and B). Glucose-stimulated insulin and total Glp-1 secretion was significantly enhanced with 264W94 treatment at both 0.1 and 10 mg/kg doses (Fig. 3, C–F).

Changes in intestinal and hepatic gene expression. To further explore potential mechanisms for 264W94-mediated antidiabetic effects, in a separate study (study 2), we examined the expression of a group of genes in the liver and intestines of ZDF rats following treatment with 264W94 for 5 wk. Consistent glucose-lowering effects were observed through the entire study duration in 264W94-treated ZDF rats (data not shown). Treatment with 264W94 had no significant effects on Fxr expression in the intestines (Fig. 4A). Hepatic Fxr mRNA was lower in ZDF rats than in lean controls and was upregulated by the treatment of 264W94 (Fig. 4A). However, hepatic small heterodimer partner (Shp) expression was reduced in 264W94-treated rats (Fig. 4B). The expression of Shp mRNA was also decreased in duodenum, jejunum, and ileum but was slightly increased in the ascending colon with 264W94 treatment (Fig. 4B). ZDF rats had reduced mRNA levels of Tgr5 in duodenum and jejunum compared with the lean controls, and 264W94 tended to further reduce the expression of Tgr5 in the small intestines but slightly increased Tgr5 mRNA levels in the large intestines (Fig. 4C). Expression of proglucagon was increased in jejunum but decreased in the distal colon with 264W94 treatment (Fig. 4D). Although not statistically significant, treatment with 264W94 lowered the expression of two major gluconeogenic enzymes, G6pc and phosphoenolpyruvate carboxykinase (Pepck) (Fig. 4, E and F) in the liver but not the intestines. The expression of fibroblast growth factor (Fgf)15 was mainly restricted to the ileum. ZDF rats had significantly lower Fgf15 expression in the ileum compared with the lean controls, and 264W94 had no effect. Although Fgf15 mRNA was barely detectable in the ascending colon of ZDF and Zucker lean control rats, 264W94 slightly but significantly increased Fgf15 expression in the first part of the colon (Fig. 4G).

Assessment of the role of fxr in the antidiabetic efficacy of 264W94. The decreased expression of Shp in the small intestine and the liver of 264W94-treated rats suggested reduced Fxr activity. We explored whether the reduction of Fxr activity contributed to 264W94-induced glycemic control by coadministration of GW4064, a potent Fxr agonist (study 3). Treatment of 264W94 (1 mg/kg) lowered blood glucose in all treated rats within 2 days, and that effect was not attenuated by adding GW4064 (30 mg/kg) as a cotreatment (Fig. 5).

DISCUSSION

The present study is the first to report an antidiabetic effect of a potent inhibitor of ileal bile acid transport (ASBTi). The progressive dysglycemia characteristic of ZDF rats, as reflected by nonfasting plasma glucose and Hb A1c measurements, was dose-dependently arrested and partly normalized with chronic oral administration of 264W94.

In contrast to the characteristic (13) progressive insulin decline in vehicle-treated ZDF rats, suggestive of B-cell failure, the effects of 264W94 on glycemic control were associated with dose-dependent preservation of plasma insulin concentration. Similarly, elevated proinsulin/insulin ratios [indicative of reduced insulin-secretory capacity, relative to demand (31)] were dose-dependently normalized with 264W94. In addition, treatment with 264W94 also dose-dependently elevated nonfasting plasma total Glp-1 concentrations. Our preliminary data suggested that it was not a direct effect of the compound on endocrine L cells, because Asbt was not expressed in GLUTag and STC-1 cells and 264W94 (up to 10

Fig. 1. Effects of 264W94 on fecal bile acid and NEFA concentrations and plasma bile acid concentrations. Male ZDF rats (n = 8) were orally administered vehicle or 264W94 twice a day for 2 wk. Fecal samples were collected for 24 h at the end of the 2nd wk of treatment. Concentrations of bile acids (A) and NEFA (B) are normalized to vehicle-treated group. C: plasma bile acid levels were determined at the end of the 2nd wk under nonfasting condition. Data are expressed as means ± SE.
mg/kg) did not induce Glp-1 release in fasted rats (unpublished data).

Dose-dependent effects on nonfasting concentrations of plasma glucose, insulin, and Glp-1 with 2-wk chronic 264W94 administration were mirrored during an oral glucose challenge. Reductions in post-challenge glucose profiles were associated with dose-dependent increases in insulin and total Glp-1 profile. These findings suggest a mechanistic association between

Fig. 2. Effects of 264W94 on plasma glucose, insulin, proinsulin, Hb A1c, and total glucagon-like peptide-1 (Glp-1). Male ZDF rats (n = 8) were administered vehicle or 264W94 (0.001, 0.01, 0.1, 1, and 10 mg/kg) twice a day for 2 wk. Plasma glucose (A), insulin (E), and total Glp-1 (G) and levels were monitored weekly. Blood Hb A1c (C) and plasma proinsulin (F) were measured before and at the end of 2 wk of treatment (C). The 2nd-wk dose response curves for glucose (B), Hb A1c (D), insulin (F), and total Glp-1 (H) were also presented. Data are expressed as means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle group.
ASBTi-mediated stimulation of Glp 1, its recognized antidiabetic effects (including stimulation of insulin secretion) (3), and the antidiabetic effect observed in the present study. However, it is important to notice that the increase of plasma Glp-1 after 264W94 treatment was significantly smaller compared with pharmacological treatment with Glp-1 analogs/mimetics. This may suggest that nutrient-induced incretin release and its effect on glucose metabolism can act through certain local gastrointestinal mechanisms that are different from those of exogenous Glp-1 analogs/mimetics. In a recent study, Waget et al. demonstrated the local importance of endogenous incretins by inhibiting dipeptidyl peptidase IV only in the gastrointestinal system (39). The fact that at 0.1 mg/kg, an efficacious dose for glucose lowering, 264W94 only increased nutrient stimulated dynamic elevation but not the steady-state plasma Glp-1 also suggested that timing could be an important factor for the glucose-lowering effect of endogenous Glp-1. Although 264W94 was a systemically available compound, we were confident that the antidiabetic effect originated from its inhibition on Asbt within the intestine for the following reasons. First, 264W94 was at least 100-fold more potent for Asbt vs. the hepatic Na^+/H^-taurocholate cotransporting polypeptide (Ntcp, unpublished data). In addition, we also tested other luminal restricted Asbt inhibitors (~100% fecal recovery of compounds) and obtained essentially the same glucose-lowering efficacy as 264W94 (unpublished data).

Asbt inhibitors block enterohepatic recirculation and deliver more free bile acids into the colon. An effect of bile acids on stimulating Glp 1 secretion is supported by several lines of evidence. In the 1980s, before the therapeutic significance of Glp-1 was understood, intraluminal application of bile acids into distal intestine stimulated the secretion of gut glucagon-like immunoreactive materials (enteroglucagon, corresponding to Glp-1) in dogs (25, 26) and healthy human subjects during colonoscopy (1). These findings were affirmed in isolated rabbit colon (4) and rat colon preparations (28). Recently, rectally administered taurocholate stimulated vigorous release of peptide YY and Glp 1 in T2D patients in association with increases in insulin and lowering of glucose that surpassed effects reported for injected Glp 1 agonists (2). In addition to direct stimulatory effects, our unpublished preliminary data also suggested that bile acids might potentiate nutrient-induced Glp-1 release, consistent with a recent report (30).

How bile acids induce Glp-1 release from enteroendocrine L cells is not clear. Katsuma et al. (17) have reported that bile acids stimulated Glp-1 secretion in a murine enteroendocrine cell line.

Fig. 3. Oral glucose tolerance test. Male ZDF rats (n = 8) were administered vehicle or 264W94 (0.1 and 10 mg/kg) twice a day. Cannulation of jugular vein was performed at the beginning of the 3rd wk. One week after surgery, an oral glucose tolerance test (OGTT) was performed using 2 g/kg dextrose. Blood glucose was measured using glucometers at 0-, 5-, 10-, 15-, 30-, 45-, and 60-min time points. Plasma samples were collected at the same time points for insulin and total Glp-1 measurements. Data are expressed as means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle group.
Fig. 4. Eight-week-old male ZDF rats were randomized on fed glucose and assigned to vehicle or 1 mg/kg bid 264W94 groups. A group of age-matched Zucker lean control rats was also included in the study. All animals were monitored for 5 wk. Treatment with 264W94 decreased serum glucose and blood Hb A1c through the study (data not shown). At the end of the study, intestinal and liver tissues were collected in the fed state, and mRNA expression of selected genes was determined using RT-qPCR. Expression level was normalized for a group of housekeeping genes. Data are expressed as means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001 vs. ZDF vehicle group.
nonfasting plasma glucose was measured using glucometers.  

Fig. 5. Assessment of the role of (farnesoid X receptor Fxr) in 264W94-induced glycemic control. Male ZDF rats were randomized on fed glucose and assigned to 4 groups (n = 6): vehicle, 264W94 (1 mg/kg bid), and 264W94 (1 mg/kg bid) + GW4064 (30 mg/kg bid). Animals were treated for 2 days, and nonfasting plasma glucose was measured using glucometers.

STC-1, via Tgr5 signaling. Using a synthetic bile acid-derived Tgr5 agonist, Thomas et al. (37) confirmed the Tgr5-dependent Glp-1 stimulatory effect both in vitro and in vivo. In a recent study, Parker et al. (27) demonstrated that Tgr5 is a molecular mechanism for bile acid-induced Glp-1 release in L cells. Chronic treatment of ZDF rats with 264W94 tended to decrease Tgr5 expression in jejunum but increase its expression level in the ascending colon. Yet, in OGTT of the present study, the fact that plasma Glp-1 increased within 5 min after glucose administration suggested an L cell-potentiating effect of bile acids. More studies are needed to demonstrate that Tgr5 agonism can both directly stimulate and modify nutrient-induced Glp-1 secretion from L cells or that additional bile acid-related mechanisms other than Tgr5 were involved in Asbt inhibition-mediated glucose-lowering efficacy. In addition to functionally stimulating Glp-1 release, inhibiting Asbt might also change the total number of L cells or the expression of proglucagon in enteroendocrine cells. While preliminary histology results did not suggest a clear increase in Glp-1-positive cells in the intestine (data not shown), mRNA analysis indicated an increase of proglucagon expression only in the jejunum. More studies are needed to determine the significance of that change.

Fxr, a nuclear receptor for bile acids, is also highly expressed in the intestine, especially in the ileum and ascending colon. Treatment with 264W94 decreased Fxr activity (as measured by Shp expression) in the duodenum and the jejunum but might have increased Fxr activity in the ascending colon (as measured by Shp and Fgf15 expression). However, administration of GW4064, a potent Fxr agonist, directly to the proximal colon did not induce Glp-1 release (data not shown). Fgf19 is a member of the fibroblast growth factor (FGF) family, and Fgf15 is the rodent ortholog of human FGF19. Bile acids stimulate Fgf19/15 release from ileum by an Fxr-dependant transcription mechanism (34). Transgenic mice expressing human Fgf19 displayed increased metabolic rate and decreased adiposity (38) and exogenous Fgf19 increased metabolic rate and reversed dietary and leptin-deficient diabetes (15). Fgf15, predominantly expressed in rat ileum, was significantly downregulated in ZDF rats and was not increased by treatment with 264W94. Although there was a significant increase of Fgf15 mRNA in the ascending colon of 264W94 treated ZDF rats, the magnitude was very modest compared with the normal level in the ileum. We consider it improbable that antidiabetic effects of Asbt inhibition were mediated via circulating Fgf15 in the current study.

Although Fxr agonism may decrease serum glucose by reducing the expression of hepatic gluconeogenesis genes and hepatic lipogenesis in multiple animal models (7, 21, 36, 42), hepatic Fxr activity, as measured by expression of Shp, was decreased by 264W94 in the current study. A recent study by Prawitt et al. (29) demonstrated that Fxr deficiency improved glucose homeostasis and insulin sensitivity in a genetically obese mouse model, and that effect was independent of bile acid-mediated pathways. Since the reduced Fxr activity observed in our study was a consequence of decreased bile acid stimulation, it was unlikely that the hypoglycemic effect of Asbt inhibition could be explained by Fxr “deficiency”. Furthermore, an Fxr agonist, GW4064, did not affect the antidiabetic effect of Asbt inhibition.

Overall, further studies are needed to understand how Asbt inhibition improves glucose metabolism, which may include mechanisms within and out of the gastrointestinal tract and are Glp-1 dependent or Glp-1 independent (30). Our unpublished preliminary results also suggest that Asbt inhibition may affect plasma and fecal bile acid composition and release of gastrointestinal hormones, nutrient absorption (e.g., NEFA) and have potential effects on gut microbiota, all of which may have an impact on glucose metabolism (10, 41). Previously, we reported similar antidiabetic effects of a BAS, cholestyramine, in ZDF rats. Since both bile acid sequestration and Asbt inhibition increased the presence of the bile acids in the distal intestine, they might share similar mechanisms for their antidiabetic efficacy. In a recent paper, Shang et al. (33) reported that colestevolam (a BAS) had an effect on a glucose tolerance test but not SC-435, an Asbt inhibitor. In contrast to the present study, Shang et al. did not assess the effects of or dose dependency of SC-435 on increasing intraluminal (or fecal) bile salt concentrations. They surmise in their discussion that they may not have achieved sufficient luminal concentrations to evoke an effective L cell response. Another notable difference is that their animal model, a diet-induced obesity (F-DIO) rat, was not a true diabetic animal model. In addition, differences in dosing regimens (oral gavage vs. food supplement) might also have an impact on outcomes.

In summary, we describe inhibition of Asbt as a potential novel pharmaceutical approach for glycemic control in T2DM. This approach is possible with agents that remain within the gut lumen, thereby posing a reduced risk of systemic toxicity. Since inhibition of Asbt also reduces low-density lipoprotein cholesterol (LDL-C) (13), it may convey a dual benefit, controlling both hyperglycemic and hypercholesterolemic cardiovascular risk in T2DM patients.

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DISCLOSURES

AUTHOR CONTRIBUTIONS


REFERENCES


