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Selective peroxisome proliferator-activated receptor-α modulator K-877 regulates the expression of ATP-binding cassette transporter A1 in pancreatic beta cells

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Selective peroxisome proliferator-activated receptor-α modulator K-877 regulates the expression of ATP-binding cassette transporter A1 in pancreatic beta cells

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ABSTRACT

ATP-binding cassette transporter A1 (ABCA1) protein is a pivotal regulator of cholesterol and phospholipid efflux from cells to high-density lipoprotein (HDL) particles. Pancreatic ABCA1 functions in beta cell cholesterol homeostasis and affects insulin secretion. We investigated the effect of pemafibrate (K-877), a novel selective PPARα modulator (SPPARMα), on pancreatic ABCA1 expression. *In vivo* experiment, mice were divided into four treatment groups, namely, normal food plus placebo, high fat diet (HFD) plus placebo, normal food plus K-877 (0.3 mg/kg/day), or HFD plus K-877 (0.3 mg/kg/day), and treated for eight weeks. The results in vitro experiment indicate that K-877 treatment increased levels of ABCA1 mRNA, as well as protein, subsequently reduced the cellular cholesterol content in INS-1 cells. PPARα specific antagonist GW6471 attenuate K-877 induced ABCA1 expression in INS-1 cells. ABCA1 promoter activity increased with K-877 treatment at concentration 1 μM and 10 μM. Glucose-stimulated insulin secretion was ameliorated by K-877 treatment in INS-1 cells and isolated mouse islets. Although the expression of ABCA1 was reduced in mice with HFD treatment, both ABCA1 protein and mRNA levels were increased in mice with K-877 treatment. K-877 treatment improved glucose intolerance induced by HFD in mice. These findings raise the possibility that K-877 may affect insulin secretion by controlling ABCA1 expression in pancreatic beta cells.

1. Introduction

The nuclear receptor superfamily, peroxisome proliferator-activated receptors (PPARs) is further divided into three subtypes: PPARα, PPARβ/δ and PPARγ. PPARα, activated by the fibrate class drugs (fibrates), is ubiquitously expressed (Dreyer et al., 1992). K-877, a newly developed selective PPARα modulator (SPPARMα), is a next generation lipid-lowering drug. K-877 shows greater PPARα activation ability than other fibrates, with a higher degrees of subtype selectivity (> 1000-fold subtype selectivity) and a lower EC50 value (Raza-Iqbal et al., 2015). K-877 has been shown to decrease serum triglyceride (TG) levels at doses ranging from 0.05 to 0.4 mg/day for 12 weeks. This reduction is equal to or greater than that reported for fenofibrate at a dose of 100 mg/day, with fewer associated side effects (Fruchart, 2017).

PPARα is expressed in the liver, heart, skeletal muscle (Pyper et al., 2010), and also in pancreas (Holness et al., 2003; Koh et al., 2003). Although PPARα ligands, such as fibrates, are used to treat patients with dyslipidemia, PPARα activation may also affect insulin secretion. Rat pancreatic islets treated with FFA showed a decrease in both PPARα expression and glucose-stimulated insulin secretion (GSIS)(Fatehi-Hassanabad and Chan, 2007). Sun et al. reported that PPARα ligand may enhanced GSIS via upregulation of pancreatic/pancreatic duodenal homeobox-1 (PDX1)(Sun et al., 2008), However, the mechanisms by which PPARα agonists impact glucose metabolism have not yet been completely elucidated.

Dyslipidemia has emerged as a contributor to pancreatic beta-cell dysfunction (Kahn, 2003). ATP-binding cassette protein A1 (ABCA1) is a critical regulator of cholesterol and phospholipid efflux from cells to

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HDL and is required for reverse cholesterol transport (Knight et al., 2003). recent report showed that mice lacking ABCA1 in their beta cells exhibited impaired glucose tolerance and decreased insulin secretion but normal insulin sensitivity (Brunham et al., 2007). These results indicate that cholesterol accumulation might induce beta-cell dysfunction in type 2 diabetes mellitus, a process known as pancreatic lipotoxicity.

Clinical studies have shown an association between fibrates and diabetes (Harrold et al., 1969; Scott et al., 2009). However, little is known about the direct effect of K-877 on pancreas-specific genes. Here, we evaluate the role of K-877 in regulating the expression of ABCA1 and how it functions in pancreatic lipotoxicity and diabetes.

2. Materials and methods

2.1. Cell culture

The INS-1 cells originated from a rat insulinoma cell line developed and propagated at the Division of Biochimie Cliniqe (courtesy of C. B. Wollheim, Geneva, Switzerland). These cells were cultured in RPMI-1640 media (SIGAMA, Tokyo, Japan) containing 11.2 mmol/l glucose and supplemented with 10% heat-inactivated fetal bovine serum (Dainippon Pharmaceutical Co., Tokyo, Japan), 50 μmol/l 2-mercaptoethanol, 100 U/ml penicillin, and 0.1 mg/ml streptomycin in a humidified atmosphere containing 5% CO2 at 37 °C. After 6 h of starvation, the cells were treated with varying doses of K-877 for 24 h before harvesting for protein extraction, mRNA extraction and cholesterol measurement.

2.2. Animals

Six-week-old male C57BL/6 J mice were used in this study. The mice were divided into four groups and treated with K-877 for 8 weeks. The normal food plus placebo group was given normal food and placebo treatment, the high fat diet (HFD) plus placebo group was given HFD and placebo treatment, the normal food plus K-877 (0.3 mg/kg/day) group was given normal food and K-877 treatment, the HFD plus K-877 group was given HFD and K-877 treatment, this concentration was adopted from the former experiment (Raza-Iqbal et al., 2015). On day 60, mice were killed under 10% chloralhydrate anesthesia (350 mg/kg ip). After scarification, pancreas was collected and stored at −80 °C until used for western blot and real-time PCR analysis. Experimental protocols and animal care methods were reviewed and approved by the Kagawa University Institutional Animal Care and Use Committee and performed according to their guidelines.

2.3. Pancreatic Islet Isolation

The pancreatic islets were removed and isolated by collagenase digestion as previously described (Lyu et al., 2016). Briefly, the pancreas was digested by collagenase XI (SIGAMA) at 37 for 15 min and then the islets were purified and collected by a Histopaque gradient. Finally, the islets (150–200 μm) were individually picked under a dissecting microscope, rinsed three times in hanks solution and cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin overnight under 5% CO2 at 37 °C. Isolated islets were used for experiments in day 3.

2.4. Glucose stimulate insulin secretion

Briefly, pancreatic islets or INS-1 cells were incubated in 10 μM K-877 or vehicle medium for 24 h, then removed to Krebs–Ringer bicarbonate (KRB) buffer and starved for 1 h at 37 °C. The Krebs–Ringer bicarbonate (KRB) buffer was supplemented with 120 mM NaCl, 5 mM KCl, 1.1 mM MgCl2, 2.5 mM CaCl2, 25 mM NaHCO3 and 0.1% bovine serum albumin (pH 7.4). Following, cells were incubated in new KRB buffer supplement with varying glucose concentration (basal: 3.3 mM, stimulatory: 16.7 mM). After incubation at 37 °C for 1 h, the supernatant was harvested and used for insulin measurement by ELISA kit (Shibayagi, Japan). All the incubation was performed in 5% CO2 incubator at 37 °C.

2.5. Real-time reverse transcriptase–polymerase chain reaction

Total cellular RNA was isolated with RNA-Bee-RNA isolation reagent, and quantified by measuring the absorbance at 260 nm. 6 μg of total RNA was used to reverse transcription. Rat ABCA1 were quantified by real time PCR. The sequences of the forward and reverse rat ABCA1 primers were 5′-CCCGGCGGAGTAGAAAGG-3′ and 5′-AGGGCGATGCA AACAAAGAC-3′. The ABCA1 were normalized to housekeeping standard Glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2.6. Western blot analysis

The 7.5% sodium dodecyl sulfate polyacrylamide gel was used to separate the proteins, then the proteins were transferred to a polyvinylidene difluoride membrane for immunoblotting. The membranes were blocked for 24 h overnight at 4 degrees with 7.5% skimmed milk in PBS supplemented 0.1% Tween 20. The blots were incubated overnight with 1:1000 diluted ABCA1 primary antibodies, followed by incubation for 1 h with a secondary antibody (HRP-conjugated anti-rabbit IgG; 1:2000) at 4 degrees. Membranes were again washed three times, 10 min each, and antigen-antibody complexes were visualized by ECL (GE Healthcare). Protein bands in Western blot analysis were obtained under Luminescent image analyzer LAS-1000Plus (Fuji Film, Japan).

2.7. Transfection and luciferase reporter assay

We used a construct (pABCA1-LUC) containing the promoter region of ABCA1. Briefly, 1 μg purified promoter plasmid was transfected into INS-1 using Lipofectamine (Life Technologies, Gaithersburg, MD). Transfected cells were treated with varying doses of K-877 for 24 h. Following, cells were harvested, and the promoter activity was measured in an aliquot of the cytoplasmic preparation. 40 μl aliquots were taken for the luciferase assay, which was performed according to the manufacturer's instructions (ToyoInk, Tokyo, Japan).

2.8. Cholesterol efflux assay

To measure cellular cholesterol ester concentration, we employed a method developed by Shahnaz et al.(Shahnaz et al., 1998), which is a completely automated fluorimetric method for the determine of cholesterol, consisting of enzymatic hydrolysis of cholesteryl ester to free cholesterol and enzymatic oxidation of free cholesterol in the presence of an indictor substrate to produce a fluorescent product.

2.9. Statistical analysis

For experiments with multiple treatments, ANOVA was used to test for differences in group means. For mouse experiments with multiple time points, we performed 2-way repeated-measures ANOVA to test for differences in means between groups. When ANOVA was significant, post hoc Bonferroni-corrected *t*-tests were applied. For comparison of data between 2 groups at a single time point unpaired *t*-tests were performed. Statistical significance is presented as $^*P < 0.05$ in all figures.

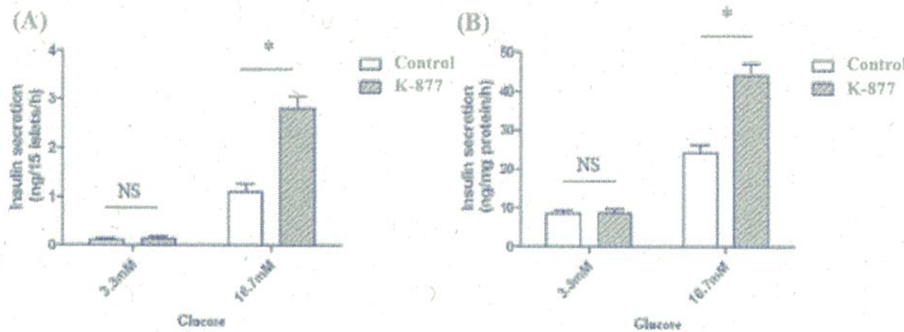

Fig. 1. The effect of K-877 on glucose-stimulated insulin secretion. A, INS-1 cells were incubated in medium containing 10 μM K-877 or the vehicle for 24 h, after which insulin secretion was stimulated by addition of low and high concentrations of glucose. B, mouse islets were isolated as described in the Methods section. Isolated islets were incubated in medium containing 10 μM K-877 or vehicle for 24 h, after which insulin secretion was stimulated by addition of low and high concentrations of glucose. Data are represented as the mean ± S.E.M. *, $P < 0.05$ vs. control.

3. Results

3.1. Effect of K-877 on glucose stimulated insulin secretion

As PPARα agonists have been shown to enhance GSIS, we examined the effect of K-877 on this process. INS-1 cells and isolated mouse islets were incubated in medium only or medium supplemented with 10 μM K-877. Insulin secretion was then stimulated with low (3.3 mM) and high (16.7 mM) concentrations of glucose, after which the insulin concentration in each medium was measured by ELISA. As shown in Fig. 1, K-877 treatment in both isolated islets and INS-1 cells significantly increased insulin secretion in response to high glucose stimulation.

3.2. Effect of K-877 on ABCA1 expression in isolated mouse islets

To determine the direct effect of K-877 on pancreatic ABCA1 expression, we isolated mouse islets as described in Methods. First, we extracted total protein in isolated mouse islets treated with K-877 at varying concentrations (0–10 μM) and used Western blotting to monitor the ABCA1 expression. We found that K-877 increased ABCA1 expression (Fig. 2A) in isolated mouse islets. Because K-877 at a concentration of 10 μM efficiently increased the ABCA1 expression, we used this concentration of K-877 in the following experiments. Then isolated islets were exposed for 24 h to unenhanced medium or medium supplemented with 10 μM K-877. ABCA1 protein levels were then measured by western blot. As shown in Fig. 2B, ABCA1 protein levels were significantly increased in isolated mouse islets, following treatment with K-877.

3.3. Effect of K-877 on ABCA1 expression and cholesterol content in INS-1 cells

To test the effects of K-877 on ABCA1 expression, INS-1 cells were exposed to varying concentrations (0–10 μM) of K-877 for 24 h, after which ABCA1 expression was examined by western blot and real-time PCR analysis. Fig. 3 shows that ABCA1 mRNA and protein levels were significantly increased following exposure to both 1 μM and 10 μM K-887 (Fig. 3A, B). Then we incubated INS-1 cells with both K877 (10 μM) and PPARα specific antagonist GW6471 (10 μM) for 24 h, and protein level of ABCA1 was measured by western blot. GW6471 significantly attenuate K877 increased ABCA1 expression (Fig. 3C). Subsequently, we tested the effect of K-877 on ABCA1 promoter activity by a luciferase reporter gene assay. A significant increase in luciferase activity was again observed following treatment with 1 μM and 10 μM K-877 (Fig. 3D). To further confirm these findings, we measured the cholesterol ester content in the cells treated with K-877 as described in the Methods section. As predicted, when INS-1 cells were exposed to varying doses of K-877, the cholesterol ester content was significantly reduced at concentrations ranging from 1 to 10 μM (Fig. 3E).

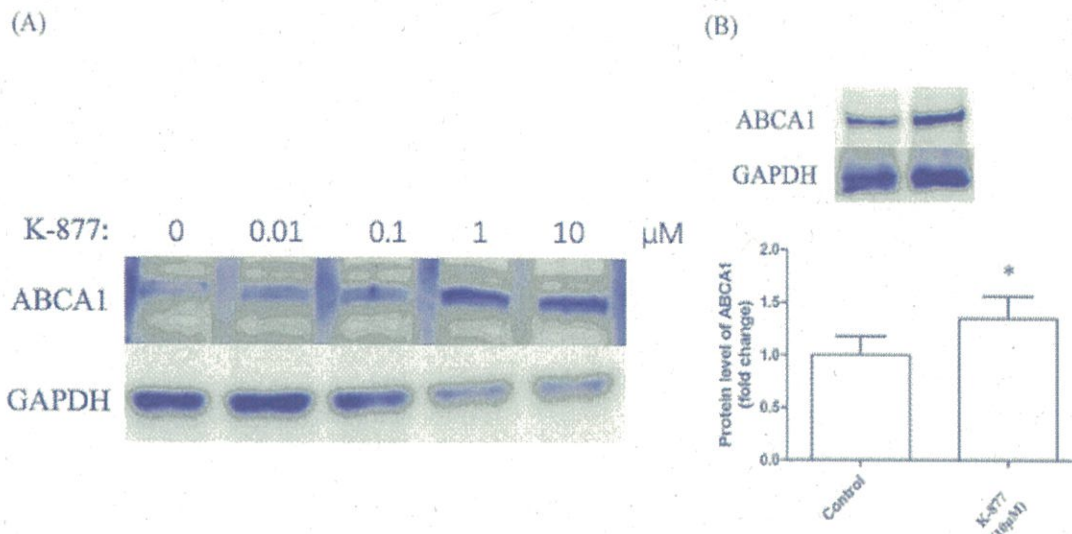

Fig. 2. The effect of K-877 on ABCA1 expression in isolated mouse islets. A, the abundance of ABCA1 protein in isolated mouse islets treated with varying concentrations (0, 10 nM, 100 nM, 1 μM, and 10 μM) of K-877. B, the abundance of ABCA1 protein in isolated mouse islets treated with K-877 at 10 μM. Data are represented as mean ± S.E.M. *, $P < .05$ vs. Control.

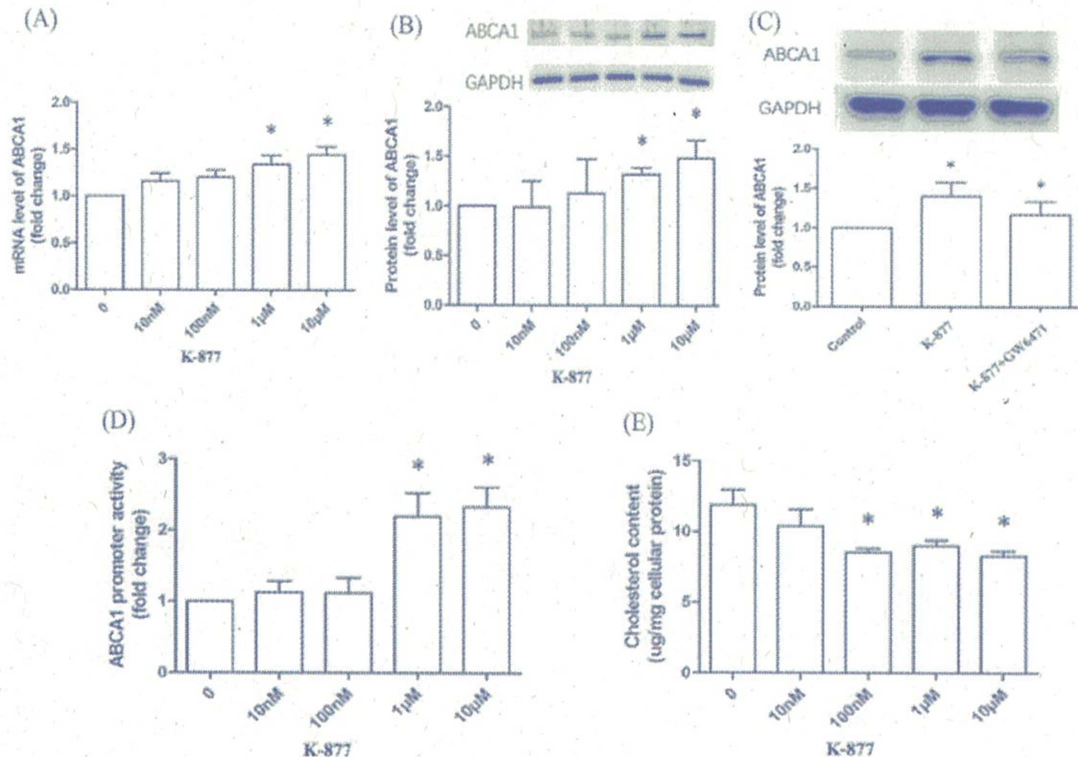

Fig. 3. The effect of K-877 on ABCA1 expression in INS-1 cells. A, ABCA1 mRNA expression. Total RNA was extracted from INS-1 cells treated with various concentrations of K-877 for 24 h, after which ABCA1 mRNA expression was analyzed by real-time PCR. All data were normalized to GAPDH. B, ABCA1 protein expression. Total protein was extracted from INS-1 cells treated with various concentrations of K-877 for 24 h, after which the ABCA1 protein expression levels were analyzed by western blot. All data were normalized to GAPDH. C, Total protein was extracted from INS-1 cells treated with 10 μM GW6471 and 10 μM K877 for 24 h, after which the ABCA1 protein expression levels were analyzed by western blot. All data were normalized to GAPDH. D, ABCA1 promoter activity. INS-1 cells were treated with various concentrations of K-877 for 24 h before cell harvesting. ABCA1 gene promoter activity was assayed, as described in the Methods section. The results of the 10 nM K-877 group, the 100 nM group, and the 1 μM K-877 group were normalized to those of the control group. E, Cholesterol ester content. INS-1 cells were exposed to various concentrations of K-877 for 24 h, at which time cholesterol content was measured as described in the Methods section. Data are represented as mean ± S.E.M. *, $P < .05$ vs. concentration 0.

3.4. Effect of K-877 on glucose tolerance in mice with HFD

In week 4, the HFD-fed mice gained more body weight compared to the mice fed normal food (Fig. 4A). As predicted, the average body weight of mice subjected to K-877 treatment was significantly lower compared to that of HFD-fed mice. To confirm the effect of K-877 on islet function, we monitored the fasting blood glucose during treatment with K-877 and employed an oral glucose tolerance test at the end of the 8-week experimental period. The fasting blood glucose gradually increased after 3 weeks of HFD intake (Fig. 4B); however, K-877 treatment significantly ameliorated this effect. A significant difference in glucose tolerance was observed for HFD-fed mice compared to that in mice fed normal food, and K-877 treatment significantly improved HFD induced glucose tolerance. (Fig. 4C).

3.5. Effect of K-877 on pancreatic ABCA1 expression in mouse

Pancreatic tissue was harvested from the mice, after which ABCA1 expression was measured by western blot analysis and real-time PCR. As shown in Fig. 5, although both the protein and mRNA levels of ABCA1 were lower in HFD-fed mice than those in mice fed normal food, K-877-treatment significantly increased ABCA1 levels compared to those measured for HFD-fed mice and the control mice fed normal food.

3.6. Effect of K-877 on pancreatic PPARα expression in mouse

As K-877 is known to function as a PPARα modulator, we next analyzed PPARα expression in those mice. Fig. 6 shows that HFD reduced PPARα expression in the mouse pancreas. In contrast, K-877 treatment increased PPARα expression compared to that in HFD-fed mice and those fed normal food.

4. Discussion

In the present study, we found that K-877 stimulates expression of ABCA1 in the pancreatic beta cell line INS-1, as well as in isolated mouse islets (Fig. 2, Fig. 3A, B, C, D). As is the case for other PPARα agonists, the structure of the K-877 protein contains an acidic region, but, in addition, benzoxazole and phenoxyalkyl side-chains are present, resulting in strongly enhanced PPARα selectivity and activity (Fruchart, 2017). In vitro assays demonstrated that K-877 is > 2500-fold more potent than the active metabolite of fenofibrate as well as fenofibric acid for enhancing the activity of human PPARα (Fruchart, 2017). Previous work identified genes regulated by K-877, both across species as well as in certain species only, by using cluster analysis (Raza-Iqbal et al., 2015). The profiles of genes regulated by K-877 and fenofibrate were compared to examine the influence of different SPPARMα on gene expression in liver. K-877 (but not fenofibric acid) significantly induces the expression of several genes in human hepatocytes, including that of mannose-binding lectin 2 (MBL2), which is involved in the regulation of the innate immune system and vascular complications in diabetes, glutamyl aminopeptidase (ENPEP), which functions in blood pressure regulation, as well as ABCA1 (Fruchart, 2017). Upregulation of genes by K-877 stimulation mostly occurs through PPARα activation, in this

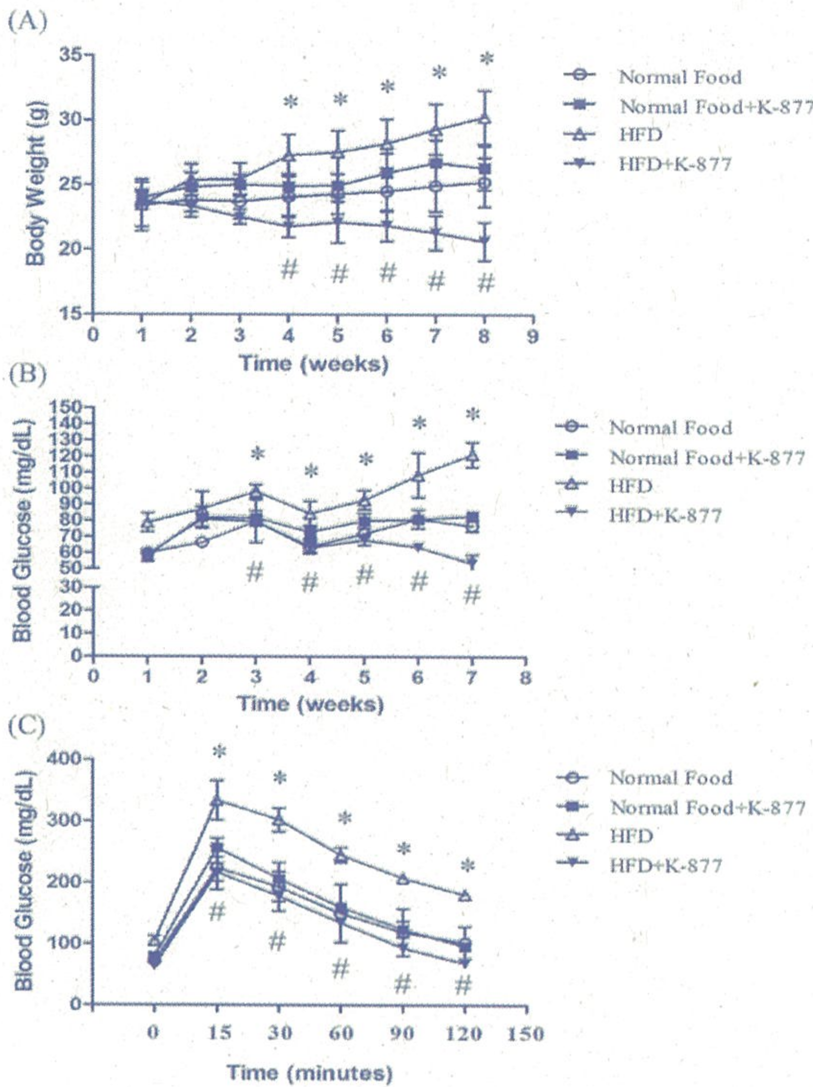

Fig. 4. K-877 improves HFD-induced obesity, hyperglycemia and glucose tolerance. A, Mouse body weights were recorded weekly. B, Mouse fasting blood glucose was measured once every two weeks. C, Oral glucose tolerance test results. Mice were fasted for 6 h. Glucose (1 mg/g) was then administered orally. Blood glucose was measured in a time dependent manner as described in the Methods section. White circles represent the normal food group (n = 5), black squares represent the normal food plus K-877 group (n = 5), white triangles represent the HFD group (n = 5), and inverted black triangles represent the HFD plus K-877 group (n = 5). Data are represented as mean ± S.E.M. *, P < .05 vs. normal food, #, P < .05 vs. HFD.

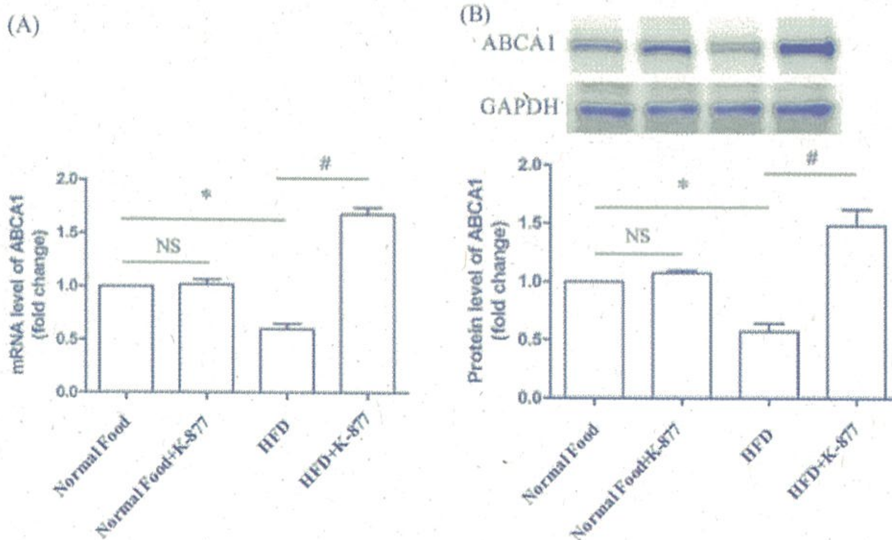

Fig. 5. The effect of K-877 on ABCA1 expression in mouse pancreas. A, ABCA1 mRNA expression. Total RNA was extracted from mouse pancreatic tissue. ABCA1 mRNA expression was analyzed by real-time PCR, and all data were normalized to GAPDH. B, ABCA1 protein expression. Total protein was extracted from mouse pancreatic tissue. ABCA1 protein expression was analyzed by western blot, and all data were normalized to GAPDH expression. Data are represented as mean ± S.E.M. *, P < .05 vs. normal food, #, P < .05 vs. HFD.

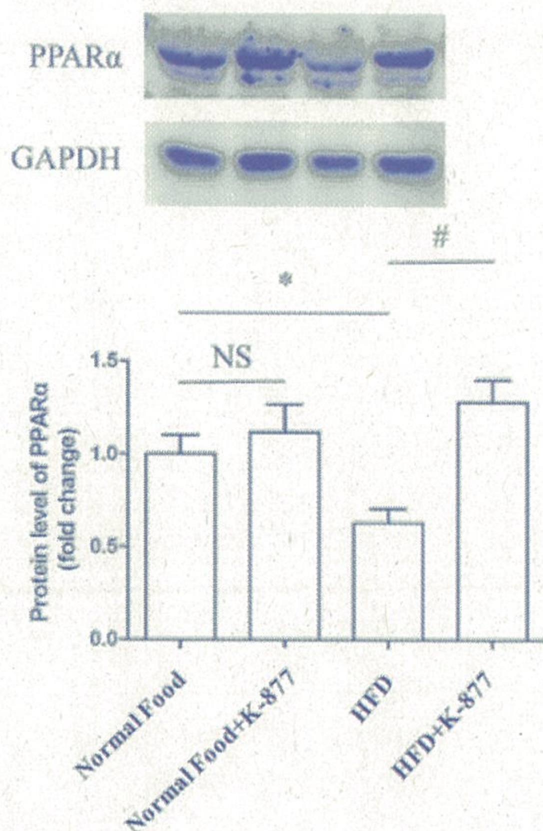

Fig. 6. The effect of K-877 on PPARα expression in mouse pancreas. Total protein was extracted from mouse pancreatic tissue. PPARα protein expression was analyzed by western blot, and all data were normalized to GAPDH expression. Data are represented as mean ± S.E.M. *, $P < 0.05$ vs. normal food, #, $P < 0.05$ vs. HFD.

study, we demonstrated that PPARα specific antagonist GW6471 attenuate K877 enhanced ABCA1 expression in INS-1 cells. Furthermore, K-877 induces expression of fibroblast growth factor 21 (FGF21) to a greater extent than fenofibric acid (Fruchart, 2017). FGF21 has been implicated in the regulation of glucose, lipid and energy homeostasis in humans. FGF21 increases HDL-mediated cholesterol efflux in oxidized low-density lipoprotein (ox-LDL)-stimulated macrophages, potentially through LXRα-dependent upregulation of ABCA1 expression (Lin et al., 2014). In this study, we determined that K-877 stimulates both protein and mRNA expression, as well as promoter activity of the ABCA1 gene in pancreatic beta-cells. Induction of ABCA1 by K-877 reduces the cholesterol content and may contribute to reduced lipotoxicity in pancreatic beta-cells. K-877 also impacts ABCA1 expression in mice fed a high-fat diet, but not in mice fed normal food (Fig. 5). HFD treatment in mice decreased PPARα expression in pancreas, while K-877 treatment in mice fed a HFD significantly increased PPARα expression compared to that in control mice (Fig. 6). Although a specific mechanism has not been elucidated yet, K-877 treatment might be useful for alleviation of lipotoxic conditions, such as diabetes.

Cholesterol accumulation in pancreatic islets, either in ob/ob mice, which lack ApoE and have diabetogenic obesity, or in cholesterol-overloaded beta cell lines, impairs the insulin secretion response to glucose (Maki et al., 2017). Consistently, high levels of cholesterol in pancreatic islets and reduced insulin secretion are detected in mice lacking beta cell ABCA1 (Brunham et al., 2008; Kruit et al., 2011), suggesting that cholesterol directly reduces the functionality of beta cells. In this study, we observed decreased cholesterol levels and subsequently enhanced insulin secretion following K-877 treatment (Fig. 1, Fig. 3E). The important role of beta cell ABCA1 in glucose homeostasis was further demonstrated by prior work indicating that rosiglitazone increases the expression of ABCA1 in beta cells (Brunham et al., 2007). We previously found that exendin-4, a long-acting analogue of the glucagon-like peptide 1 (GLP-1) and insulin-like growth factor I (IGF-I) also have a stimulating effect on ABCA1 expression at the transcriptional level, resulting in increased insulin secretion (Li et al., 2010).

PPARα activators have been shown to improve insulin resistance in type 2 diabetic animals and patients with insulin resistance syndrome (Koh et al., 2003; Ravnskjaer et al., 2005; Sun et al., 2008). Park et al. reported that fenofibrate dramatically improves hyperglycemia, insulin resistance, albuminuria, and glomerular lesions in db/db mice (Park et al., 2006). Multiple mechanisms have been proposed for the insulin-sensitizing effect of PPARα agonists. PPARα activators have been found to increase hepatic fatty acid catabolism, resulting in a decreased systemic and tissue free fatty acid content (Boden et al., 1994). Fibrates have also been reported to reduce the triglyceride content in skeletal muscle, which has been correlated with improved insulin sensitivity (Chalkley et al., 1998). Finally, PPARα activation suppresses monocyte production of inflammatory cytokines including interleukin-6 and tumor necrosis factor (TNF)-α, thereby improving insulin resistance (Pineda Torra et al., 1999). Koh et al. reported that fenofibrate has a protective effect on pancreatic beta cells, as it prevents an increased triglyceride content in the pancreatic islets of OLETF rats by stimulating fatty acid oxidation and decreasing plasma lipid levels (Koh et al., 2003). In contrast, fenofibrate, a PPARα agonist, potentiates GSIS under elevated palmitate conditions, possibly via the upregulation of PDX-1 expression. Although K-8–77 reduces cholesterol content and lipotoxicity in pancreatic beta cells, K-877, unlike fenofibrate, it does not appear to affect PDX-1 expression (data not shown). In another report, K-877 treatment was shown to prevent excessive oxidative stress in diabetic islets, suggesting that K-877 may exert a protective effect by inhibiting oxidative stress in pancreatic beta cells (Maki et al., 2017). Further experiments will be needed to elucidate the regulatory mechanisms involved in this process.

Tangier disease (TD) is a rare autosomal recessive disease caused by mutations in the ABCA1 gene. The deficiency of high density lipoprotein (HDL) in patients with TD leads to the accumulation of cholesterol in many tissues, such as the pancreas, spleen, tonsils, liver, gastrointestinal mucosa, lymph nodes, and peripheral nerves (Mott et al., 2000). Furthermore, ABCA1 is expressed in pancreatic beta cells (Brunham et al., 2007), suggesting that ABCA1 deficiency in conjunction with the presence of abnormal lipid rafts might result in the dysfunction of pancreatic beta cells in patients with TD. Koseki et al. reported that four Japanese TD patients who had also been diagnosed with type 2 diabetes exhibited a significantly lower value of the insulinogenic index compared to non-diabetic controls, as assessed by the oral glucose tolerance test (OGTT). This raises the possibility that ABCA1 plays an important functional role in glucose-responsive insulin secretion from beta cells (Koseki et al., 2009). In this study, we demonstrate that K-877 treatment improves glycemic control, likely through both the attenuation of lipotoxicity by through ABCA1 upregulation as well as the prevention of beta-cell deterioration in HFD treated mice.

In summary, our work shows that K-877 stimulates the expression of endogenous ABCA1 and improves glucose tolerance in pancreatic beta cells and in HFD-fed mice. These findings suggest that K-877 may affect insulin secretion by controlling ABCA1 expression in pancreatic beta cells. This raises the possibility of new therapeutic options for the treatment of lipotoxicity in diabetes mellitus.

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