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Selective Peroxisome Proliferator-Activated Receptor-α Modulator K-877 Stimulated the Expression of ABCA1 in Pancreatic Beta Cells in a Glucotoxic State

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ABSTRACT

Background: ATP-binding cassette protein A1 (ABCA1) is a critical regulator of cholesterol and phospholipid efflux from cells to HDL. Additionally, ABCA1 is important for insulin secretion, as evidenced by impaired glucose tolerance and decreased insulin secretion in mice lacking ABCA1 in pancreatic β cells. Long-term hyperglycemia in type 2 diabetes mellitus, a phenomenon that causes dysfunction of pancreatic β-cells and insulin resistance, is called "glucotoxicity" and is clinically important as it aggravates diabetes. Here, we investigated the effects of pemafibrate (K-877), a novel selective PPARα modulator, on insulin secretion under glucotoxic conditions.

Methods: The pancreatic beta cell line INS-1 cells were cultured in 11.2 mmol/l and 22.4 mmol/l glucose for 7 days and the expression of ABCA1 and CaMKIV was examined by western blotting and real-time polymerase chain reaction (PCR) methods. Furthermore, changes in cholesterol content when INS-1 cells were cultured under hyperglycaemic conditions were confirmed by Oil Red O staining. Changes in Glucose stimulate insulin secretion (GSIS) were also examined.

Results: ABCA1 was downregulated in INS-1 cells under high glucose conditions for seven days; however, K-877 upregulated ABCA1. Moreover, chronic hyperglycemia caused intracellular lipid accumulation, which was ameliorated by K-877. Similarly, K-877 increased glucose-stimulated insulin secretion, which was suppressed under high glucose conditions. Furthermore, CaMKIV expression was suppressed under chronic high glucose conditions, but improved by K-877.

Conclusions: These findings suggest that K-877 may affect insulin secretion by controlling ABCA1 expression in pancreatic beta cells in a glucotoxic state.

Keywords: Pemafibrate; ABCA1; Glucotoxicity; Insulin Secretion; CaMKIV

Abbreviations: ABCA1: ATP-Binding Cassette Protein A1; PCR: Polymerase Chain Reaction; GSIS: Glucose Stimulate Insulin Secretion; PPARs: Peroxisome Proliferator Activated Receptors; HDL: High-Density Lipoprotein; SPPARM alpha: Selective PPAR Modulator Alpha; FFA: Free Fatty Acid; CaMK: Calmodulin Kinase; CaMKIV: Calcium/Calmodulin-Dependent Protein Kinase IV; KRB: Krebs-Ringer Bicarbonate; PFA: Paraformaldehyde; PBS: Phosphate Buffered Saline; VSMCs: Vascular Smooth Muscle Cells; p38 MAPK: p38 Mitogen-Activated Protein Kinase; PGC-1α:PPAR-Gamma Coactivator-1 Alpha; RXR: Retinoid X Receptor; ER: Endoplasmic Reticulum

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Introduction

Peroxisome proliferator activated receptors (PPARs) are proteins of the so-called nuclear receptor superfamily that regulate gene transcription in response to ligands. Three subtypes of PPARs have been identified: PPARα, PPARγ, and PPARβ/δ. Among these, PPARα is activated by fibrates, and its expression is found mostly in the liver, pancreas [1], brown adipose tissue, heart, and kidney [2]. Free fatty acids and other physiological ligands activate the PPARα pathway. This activation of PPARα decreases blood triglyceride levels and increases High-Density Lipoprotein (HDL) levels. Exogenous ligands include fibrate drugs, such as bezafibrate and clofibrate, and most target genes are related to lipid metabolism and are primary targets of hypertriglyceridemia-modifying drugs. Selective PPAR modulator alpha (SPPARM alpha) has been newly developed and is a next-generation lipid-lowering agent that selectively regulates PPARα-mediated gene transcription. K-877, which is SPPARM alpha, increases the benefits and reduces the risks of conventional PPARα agonists [3]. Moreover, K-877 has a superior PPARα activation activity, higher degree of subtype selectivity (by > 1000-fold), and lower EC50 value compared with other fibrates [4]. ATP-binding cassette protein A1 (ABCA1) is an important membrane protein that is very necessary for reverse cholesterol transport [5]. ABCA1 is expressed on the plasma membrane and regulates the steps in the efflux of intracellular cholesterol and phospholipids to HDL.

Recently, Brunham, et al. [6] reported on insulin secretion and sensitivity in mice lacking ABCA1 in pancreatic beta cells. They reported that the pancreatic β cell specific ABCA1 knockout mice had normal insulin sensitivity but caused reduced insulin secretion and impaired glucose tolerance. These results suggest that cholesterol accumulation in pancreatic β cells may cause β cell dysfunction. This is known as "pancreatic lipotoxicity". We have previously reported the effects of K-877 on the expression of pancreatic β-cell ABCA1 [7]. Our results suggest that K-877 may regulate ABCA1 expression and affect insulin secretion in β cells. Another study demonstrated that treatment of rat islets with Free Fatty Acid (FFA) decreases PPARα expression and inhibits insulin secretion in response to glucose stimulation [8]. Such secretion of insulin in response to glucose stimulation is called GSIS (Glucose Stimulated Insulin Secretion). The fact that insulin secretion is altered by the expression level of PPRAα in the pancreas suggests that PPARα may play an important role with regard to the mechanism of insulin secretion in the pancreas. However, the detailed mechanisms, such as how PPARα agonists affect insulin secretion, are not fully elucidated. On the other hand, "glycotoxicity" is a very important clinical issue in diabetes. Persistent hyperglycemia causes dysfunction of pancreatic beta cells. In addition, insulin action is reduced in insulin target organs such as skeletal muscle, adipose tissue, and liver, resulting in insulin resistance.

This phenomenon is called "glucotoxicity," and is widely known clinically as a factor that aggravates diabetes. Expression of the insulin gene in pancreatic β-cells is suppressed, insulin secretion is decreased, and increased apoptosis is observed in the state of pancreatic β-cell glucotoxicity. Various mechanisms related to glucotoxicity have been reported, one of which potentially involves calmodulin kinase (CaMK). Calmodulin (CaM) is a calcium-binding protein that senses calcium levels and functions as an intermediary molecule to transmit information to various proteins. The CaMK family is one of the most well-defined families of enzymes. Among them, binding to the Ca2+/CaM complex activates calcium/calmodulin-dependent protein kinase IV (CaMKIV). In addition, it is activated by being phosphorylated by CaM kinase kinase (CaMKK) [9] and plays various roles in intracellular signalling. Previously, we investigated the relationship between glucotoxicity and CaMKIV in pancreatic β cells [10]. The expression of CaMKIV was decreased when β cells were cultured under conditions of high concentrations of glucose. This suggested that CaMKIV may be involved in glucotoxicity. In this study, we analyzed GSIS in β cells in a glucotoxic state. In addition, we examined the effect of K877 on insulin secretion and ABCA1 expression in pancreatic β cells under chronic hyperglycemic states (diabetes / glucotoxicity).

Materials and Methods

Cell Culture

INS-1 cells, which developed by the Division of Biochimie Cliniqe (courtesy of C. B. Wollheim, Geneva, Switzerland), were used in this study. The cells are derived from the rat insulinoma cell line. RPMI-1640 medium (SIGAMA, Tokyo, Japan) was used to culture these cells. This medium contained 11.2 mmol/l and 22.4 mmol/l glucose, respectively, supplemented with 10% heat-inactivated fetal bovine serum (Dainippon Pharmaceutical Co., Tokyo, Japan). In addition, 0.1 mg/ml streptomycin,100 U/ml penicillin, and 50 μmol/l 2-mercaptoethanol are added. The cells were exposed to chronic hyperglycemic conditions for 7 days at 37°C in humidified atmosphere containing 5% CO2. We changed the medium daily to keep the glucose concentration in the medium constant. For the K-877 group (K-877, provided by Kowa Company, Ltd. (Nagoya, Japan)), the cells were treated with 1 μM K-877 for 7 days and then collected for cholesterol measurement, mRNA extraction, and protein extraction.

Glucose Stimulate Insulin Secretion

Each experiment was performed using cells cultured under various conditions described below. GSIS was performed after culturing INS-1 cells at 11.2 mM, 22.4 mM and 22.4 mM+K-877 for 7 days. INS-1 cells were then withdrawn into Krebs-Ringer bicarbonate (KRB) buffer and incubated at 37°C while starved for 1 hour. The KRB buffer was supplemented with 0.1% bovine serum albumin (pH 7.4), 25 mM NaHCO3, 2.5 mM CaCl2, 1.1 mM MgCl2, 5 mM KCl and 120 mM NaCl. Cells were then incubated with a new KRB buffer supplement. This KRB buffer varied the glucose concentration as follows: stimulatory: 16.7 mM, basal: 3.3 mM. After 1 h of incubation at a controlled temperature of 37 °C, we collected the supernatant of these cells. The collected supernatant was assayed for insulin levels using an ELISA kit (Shibayagi, Japan). All incubations were performed in a 5% CO2 incubator at a controlled temperature of 37°C.

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Real-Time Polymerase Chain Reaction

RNA-Bee-RNA isolation reagent was used to isolate total cellular RNA. Absorbance at 260 nm was used to quantify this total cellular RNA. For reverse transcription, 6 μg of total RNA was used. With regard to sequence, the forward and reverse primer sequences for rat ABCA1 were 5′-CCCGGCGGAGTAGAAAGG-3′ and 5′-AGGGCGATGCAAACAAAGAC-3′. GAPDH was used as the housekeeping gene.

Western Blot Analysis

Proteins were separated by using 7.5% sodium dodecyl sulfate polyacrylamide gels. Then, the proteins were transferred to a polyvinylidene difluoride membrane for immunoblotting. 7.5% skim milk was prepared from a solution of 0.1% Tween 20 in PBS. Using this solution, the membranes were blocked overnight at a controlled temperature of 4 degrees for 24 hours. The membrane was incubated with either anti-ABCA1 antibody (1:1000; Santa Cruz), anti-GAPDH antibody (1:5,000; Biomol Research), anti-CaMKIV (1:2000; Abcam), and anti-phospho-CaMKIV Thr196 antibody (1:1000; Santa Cruz). After overnight incubation with the primary antibody, incubation was continued with the secondary antibody at 4 degrees for 1 hour (HRP-labeled anti-rabbit IgG; 1:2000). Membranes were then washed again. This was done three times for 10 minutes each. Then, ECL (GE Healthcare) was used to visualize antigen-antibody complexes. Under Luminescent image analyzer LAS-1000 Plus (Fuji Film, Japan), protein bands in western blot analysis were obtained.

Cholesterol Efflux Assay

The method reported by Shahnaz, et al. [11] was adopted to measure intracellular cholesterol ester concentrations. This method performs various reactions in the presence of an indicator substrate. This reaction enzymatically hydrolyzes cholesterol esters to free cholesterol and enzymatically oxidizes the free cholesterol. This cholesterol assay is a fully automated method that produces a fluorescent product.

Oil Red O Stain

First, INS-1 cells were seeded on coverslips and subjected to various treatments. 4% paraformaldehyde (PFA) was then used to fix the cells. This was done at room temperature and for 30 minutes. The cells were washed three times with phosphate buffered saline (PBS; pH 7.2). The fixed cells were then incubated with Oil Red O solution for 15 min. After washing three times with PBS, cells were incubated with hematoxylin solution for 30 seconds to stain nuclei. After washing three times with PBS, cells were mounted. Pictures were taken using an upright microscope (Olympus BX-51/DP-72).

Statistical Analysis

In multi-treatment experiments, group mean differences were tested by ANOVA for comparisons. An unpaired t-test was performed to compare data between the two groups. Data was expressed as mean ± SE. *P<0.05 indicates statistical significance in all figures.

Results

K-877 Enhanced the Expression of ABCA1 in INS-1 Cells

We analyzed the effect of K-877 on the expression of ABCA1. Western blotting was performed to measure the protein expression of ABCA1 in INS-1 cells. The results show that ABCA1 protein expression is reduced when INS-1 cells are cultured under high glucose conditions (Figure 1). We further examined changes in these cells when exposed to K-877 for 7 days. We have exposed cells to various concentrations of K-877 (0, 10 nM, 100 nM and 1 μM) to investigate the optimal K-877 concentration for ABCA1 expression in INS-1 cells. As exposure to 1 μM K-877 significantly increased ABCA1 expression, we chose 1 μM as the concentration of K-877 to proceed with our experiments. Then, compared to control cells, treatment with K-877 significantly increased endogenous ABCA1 protein (Figure 1). The expression of ABCA1 mRNA was also assessed using real-time PCR. The expression of ABCA1 mRNA was elevated by K-877 compared to cells cultured under glucotoxic conditions (Figure 2). Surprisingly, K-877 also stimulated the expression of ABCA1 even when cultured under non-glucotoxic condition. These results clearly show that K-877 increases ABCA1 expression not only under non-hyperglycemic conditions but also under hyperglycemic conditions.

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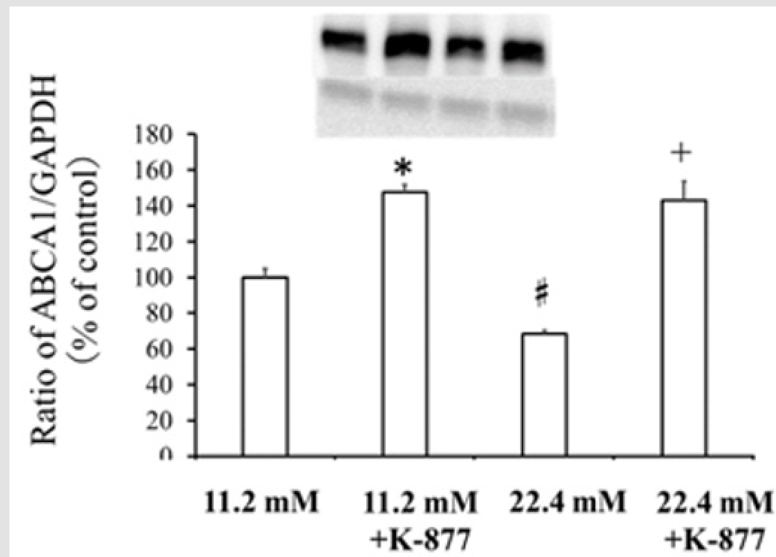

Figure 1: K-877 enhanced the protein expression of ABCA1 in INS-1 cells. Data for each treatment group represents the mean ± SEM of three separate experiments. *P < 0.05 and #P < 0.05 vs. control (11.2 mM) group; +P < 0.05 vs. control (22.4 mM) group. Whole cell extracts from INS-1 cells cultured under normal glucose conditions (11.2 mM), high glucose conditions (22.4 mM), and high glucose conditions (22.4 mM) treated with K-877 (1 μM) for seven days were examined for ABCA1 protein expression using western blotting. GAPDH was used as a control to show the ratio of ABCA1 to GAPDH as a percentage.

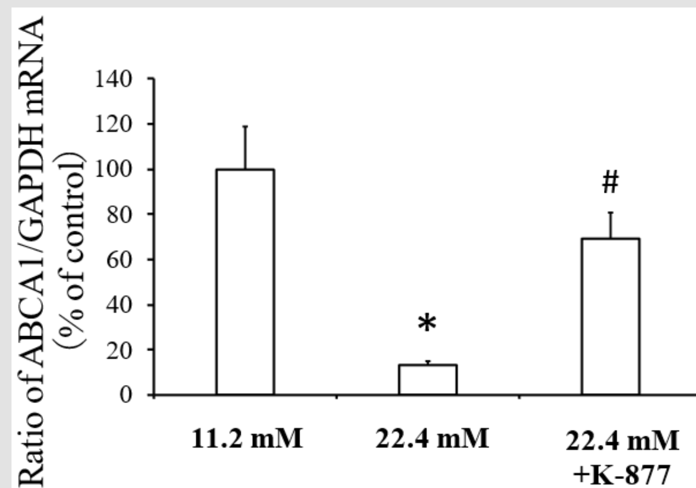

Figure 2: ABCA1 mRNA expression under hyperglycemic conditions and the effect of adding K-877. INS-1 cells were cultured for seven days under normal glucose condition (11.2 mM), high glucose condition (22.4 mM), and high glucose condition + K-877 (1 μM). We then analyzed ABCA1 mRNA expression using real-time PCR. ABCA1 mRNA expression was normalized to that of GAPDH.

Cholesterol Accumulation in INS-1 Cells was Improved by K-877

ABCA1 is an important factor for intracellular cholesterol efflux. Based on this, the accumulation of cholesterol was assessed by measuring the intracellular cholesterol content. To stain intracellular lipids such as triglycerides and cholesteryl esters in cells, we performed Oil Red O staining. Figure 3 shows that intracellular lipid droplets were increased and enlarged in INS-1 cells cultured in glucotoxic state, whereas K-877 treatment reversed this effect. It also shows that treatment of cells with K-877 results in fewer intracellular lipid droplets. In addition, the size of the lipid droplets was also reduced. Intracellular cholesterol accumulated in INS-1 cells cultured under high glucose conditions; however, addition of K-877 under these high glucose conditions reduced the intracellular cholesterol content.

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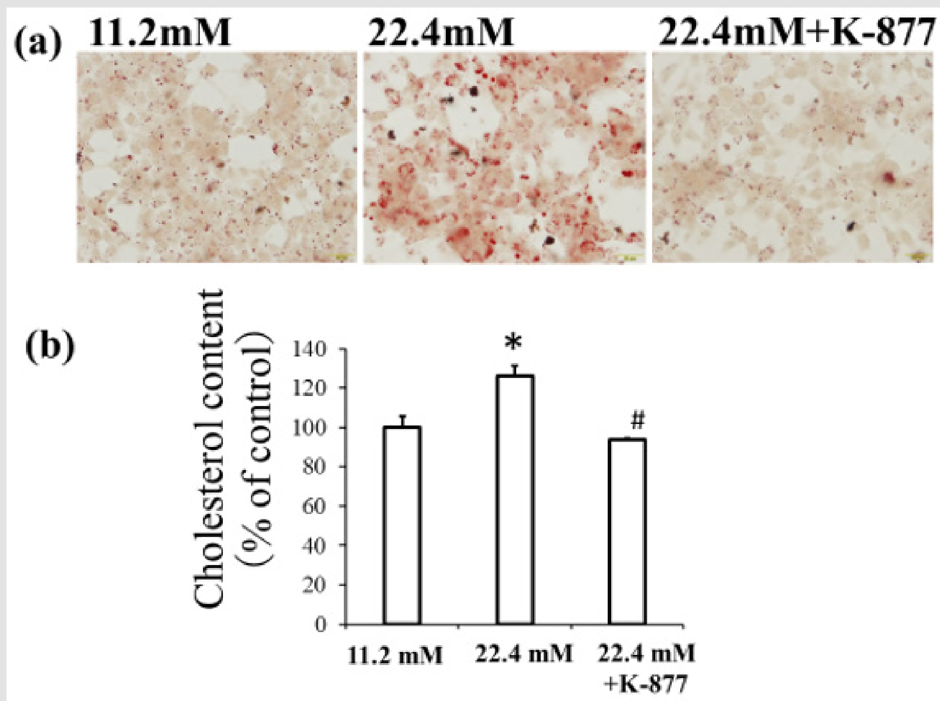

Figure 3: K-877 improved cholesterol content in INS-1 cells.

(a) INS-1 cells were cultured under the following glucose conditions; normal glucose concentration (11.2 mM), high glucose concentration (22.4 mM), and high glucose concentration + K-877 (1 μM) for seven days, and then we performed Oil Red O staining.

(b) Results of cholesterol content.

Data for each treatment group represents the mean ± SEM of three separate experiments. *P < 0.05 vs. control group; #P < 0.05 vs. 22.4 mM glucose group.

K-877 Improved Glucose Stimulated Insulin Secretion

We next investigated the effect of K-877 on GSIS under chronic high glucose conditions. INS-1 cells were first stimulated with a low glucose concentration (3.3 mM) and then with a high concentration of glucose. ELISA was used to measure insulin levels secreted from β cells into the medium. A comparison of insulin secretion from INS-1 cells in the groups incubated under normal and high glucose conditions is shown in Figure 4. Figure 4 shows that the differences in insulin levels secreted under low- and high- glucose stimulation were significantly reduced in the glucotoxic group. In particular, insulin levels did not increase in response to increasing glucose concentrations in a group of glucotoxic cells cultured at 22.4 mM. On the other hand, treatment of this glucotoxic group with K-877 clearly improved the amount of insulin secreted in response to increased glucose concentration. These K-877-induced changes were particularly pronounced at high glucose concentrations of stimulation. K-877 treatment also showed a non-significant improvement in insulin secretory capacity at low glucose concentrations (3.3 mM). These results indicate that the addition of K-877 to INS-1 cells may have a positive effect on GSIS.

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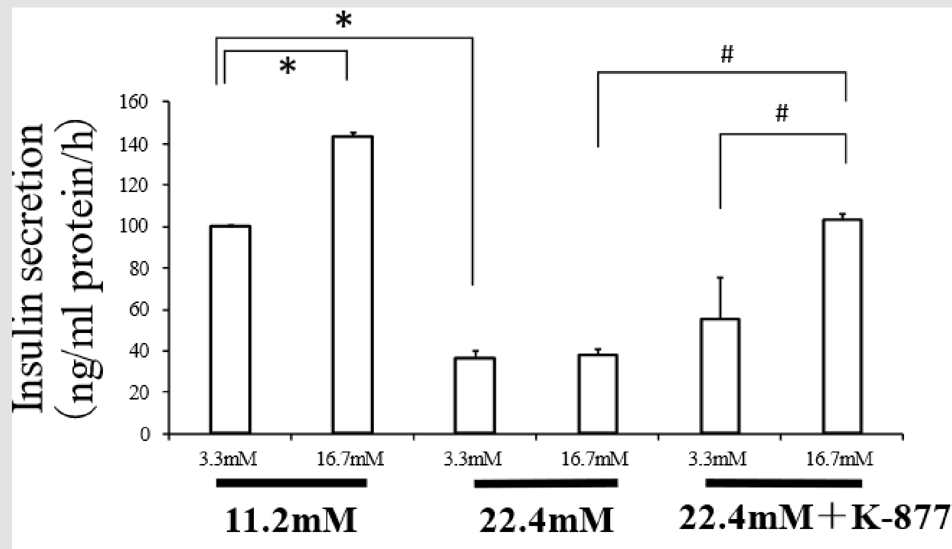

Figure 4: K-877 improved glucose stimulated insulin secretion. GSIS was confirmed after 7 days of culture of INS-1 cells. Medium containing K-877 (1 μM) or vehicle was used to culture the cells. Data for each treatment group represents the mean ± SEM of three separate experiments. *P < 0.05 vs. control (11.2 mM) group; #P < 0.05 vs. 22.4 mM group.

Effect of K-877 on the Phosphorylation of CaMKIV Under Glucotoxic Conditions

As previous reports indicated that CaMKIV is involved in the pathogenesis of glucotoxicity in pancreatic β cells [10,12], we focused on culture conditions, such as glucotoxicity, to determine whether phosphorylation of CaMKIV is altered. As represented in Figure 5, CaMKIV phosphorylation was lower in cells cultured with 22.4 mmol/L glucose for seven days than in cells cultured with 11.2 mmol/L glucose. The results so far indicate that high glucose concentration may decrease the expression of CaMKIV in pancreatic β cells. Furthermore, the expression of CaMKIV was higher in K-877-treated cells cultured under high glucose conditions for seven days than in control cells (Figures 5a & 5b). K-877 enhanced CaMKIV / phospho-CaMKIV expression regardless of glucose concentration (Figures 5a & 5b). Collectively, these results indicate that K-877 enhances the CaMKIV expression and induces the phosphorylation of CaMKIV in INS-1 cells despite high glucose conditions (Figure 6).

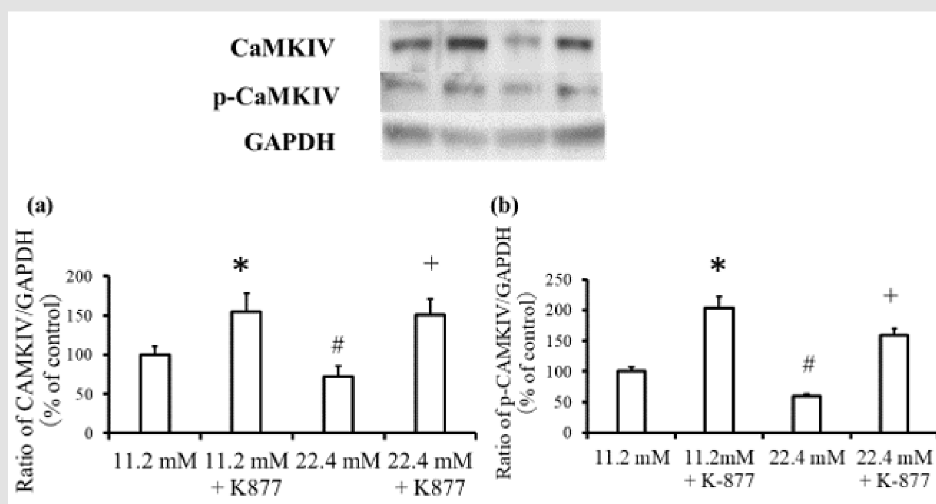

Figure 5: K-877 enhances CaMKIV / phospho-CaMKIV expression. Data for each treatment group represents the mean ± SEM of three separate experiments. *P < 0.05 vs. control (11.2 mM) group; #P < 0.05 vs. 11.2 mM group; +P < 0.05 vs. 22.4 mM group.

(a) Expression level of CaMKIV protein. INS-1 cells were treated with K-877 (1 μM) for 7 days. Proteins were extracted from these cells and analyzed for CaMKIV expression using western blotting. GAPDH was used as a control to show the ratio of CaMKIV to GAPDH as a percentage.

(b) Expression level of phospho-CaMKIV protein. INS-1 cells were treated with K-877 (1 μM) for 7 days. Proteins were extracted from these cells and analyzed for phospho-CaMKIV expression using western blotting. GAPDH was used as a control to show the ratio of phospho-CaMKIV to GAPDH as a percentage.

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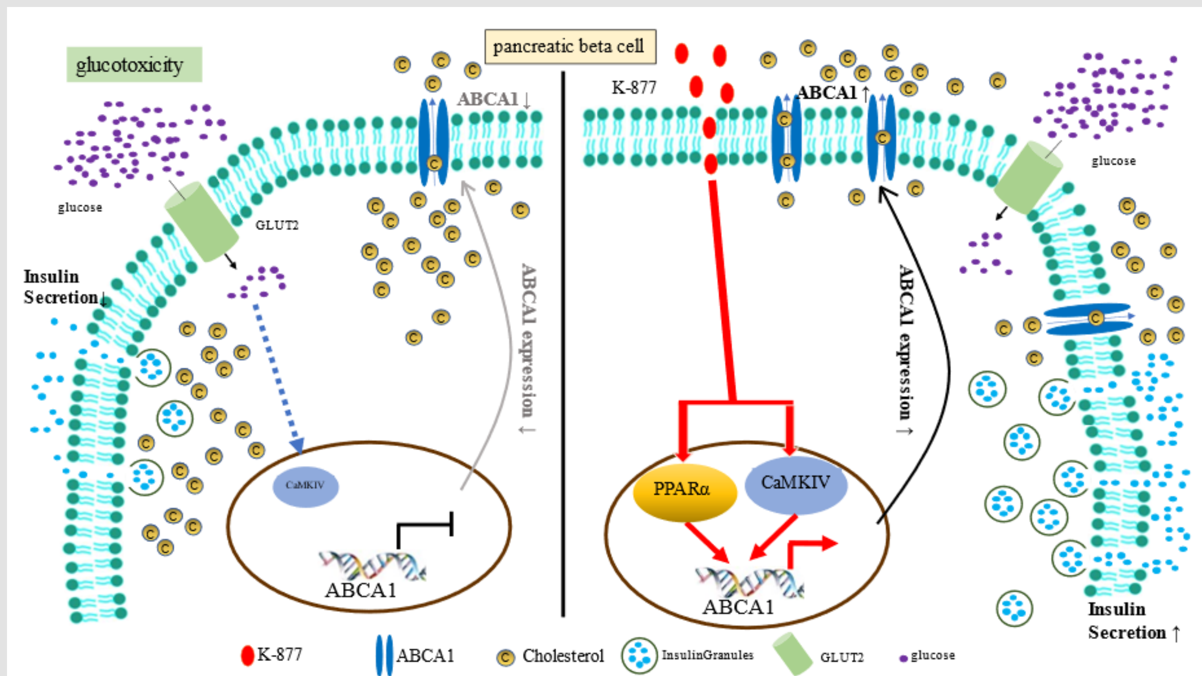

Figure 6.

Discussion

In this study, we found that exposure of INS-1, a pancreatic beta cell line, to high glucose conditions for seven days suppressed ABCA1 expression, while K-877 reversed this effect. Furthermore, chronic hyperglycemia caused lipid accumulation in INS-1 cells, as shown by cholesterol content analysis and Oil Red O staining but was ameliorated by K-877 treatment. Additionally, GSIS, which was suppressed under high glucose conditions, was ameliorated by K-877 treatment. As for the intracellular signaling pathway, the expression of CaMKIV was reduced under chronic high glucose conditions but improved by K-877 treatment. Various effects of intracellular lipid accumulation associated with ABCA1 deficiency on GSIS have been reported. Interestingly, in pancreatic beta cell selective ABCA1 knockout mice, the lipid content in pancreatic beta cells was increased and GSIS was impaired (6). Previously, we reported that TNF-α, angiotensin II, and oxidized low-density lipoprotein increase intracellular cholesterol content and reduce the expression of ABCA1 in pancreatic β cells [13-15]. We also reported that GSIS is decreased in INS-1 cells with accumulated cholesterol. In other words, lipid accumulation in pancreatic β-cells owing to decreased ABCA1 expression reduces GSIS. In a previous study, we have reported that K-877 is closely involved in the expression of ABCA1 in pancreatic beta cells. K-877 enhanced ABCA1 mRNA and protein expression by enhancing the promoter activity of the ABCA1 gene.

In addition, K-877 suppressed cholesterol content in pancreatic beta cells as a result of ABCA1 induction [7], which may contribute to decreased pancreatic beta cell lipotoxicity. In addition, the relationship between PPARα and ABCA1 was investigated. When mice were fed a high-fat diet, the expression of PPARα in the pancreas was reduced. On the other hand, administration of K-877 to mice fed an HFD restored the reduced PPARα expression. These findings suggest that PPARα may be closely related to the regulation of ABCA1 expression by the action of K-877.We have also previously reported on the relationship between Vascular Smooth Muscle Cells (VSMCs) and glucotoxicity: in VSMCs, ABCA1 expression is down-regulated by exposure to high glucose conditions [16]. In that experiment, intracellular signaling pathways were also examined. Under high glucose conditions, ABCA1 expression is suppressed. In relation to this, it was suggested that activation of the p38 mitogen-activated protein kinase (p38 MAPK) pathway may be partly responsible for this suppression of ABCA1. In our study, we found that ABCA1 expression is down-regulated in pancreatic beta cells under conditions of glucotoxicity, while K-877 induced ABCA1 expression. Previous reports showed that activation of p38 MAPK induces PPARα phosphorylation. In addition, it has been mentioned that the transcriptional activity of PPARα is inhibited by a tripartite interaction between PPARα, p38 MAPK, and ZIP/p62 [17]. Although the mechanism by which K-877 affects the suppression mechanism of PPARα by p38 MAPK activation during hyperglycemia is unknown, K-877 might affect the crosstalk of the intracellular signaling system p38 MAPK-PPARα.

Additionally, although K-877 is a selective regulator of PPARα-mediated gene transcription, this pathway functioned even under glucotoxic conditions. In this study, K-877 promoted GSIS and improved CaMKIV expression, even under glucotoxic conditions. Interesting-

ly, K-877 significantly induced CaMKIV expression at both 22.4 mM and 11.2 mM glucose concentrations in the cell culture. In addition to the improvement in CaMKIV expression, which was decreased by glucotoxicity, the possibility of K-877 having a direct effect on CaMKIV should be considered. There are various potential mechanisms responsible for the relationship between K-877 and CaMKIV. First, K-877 has been reported to inhibit cell apoptosis. Wei, et al. [18] reported that K-877 inhibits the expression of caspase and may be involved in the regulation of apoptosis in mitochondria. Sugiyama, et al. [10] reported that, under high glucose conditions, calpain, an important protease involved in apoptosis, is activated and CaMKIV expression is decreased, and this change was completely blocked by calpain inhibitors. Compared to calpain activity in INS-1 cells under normal conditions, calpain activity in INS-1 cells under glucotoxic conditions was shown to be higher. In contrast, CaMKIV was downregulated. These findings suggest that calpain is involved in the reduction of CaMKIV protein levels in glucotoxic conditions. These also emphasize the importance of calpain in the regulation of basic signaling pathways in diabetes. Based on these reports, K-877 may improve CaMKIV expression by inhibiting calpain activation.

Second, PGC-1α (PPAR-gamma coactivator-1 alpha) is a factor involved in the regulation of CaMKIV and PPARα. PGC-1α is a transcriptional coactivator that binds to various transcription factors, mainly nuclear receptors, and regulates the expression of target genes. For example, CaMKIV is activated by exercise stimulation, which in turn activates PGC-1α [19]. Furthermore, PGC-1α binds to the PPARα and RXR (retinoid X receptor) complex to affect its function [20] and is also involved in apoptosis [21]. These findings suggest that PGC-1α may play an important role in the action mechanism of K-877, which binds to PPARα and stimulates CaMKIV expression. Third, K-877 improved insulin secretion by increasing ABCA1 expression and might reduce lipotoxicity in the pancreas; however, it is also possible that the autocrine effects associated with the action of insulin itself improved insulin secretion. Leibiger, et al. [22] reported an important mechanism for insulin biosynthesis. They elucidated that in pancreatic beta cells, insulin secreted under physiological stimuli promotes transcription of the insulin gene in an autocrine fashion. They showed that secreted insulin acts on the cells themselves by mediating insulin receptors and upregulating insulin gene transcription via the CaMK pathway. This indicates that insulin acts on pancreatic beta cells to activate CaMK. Furthermore, we previously reported that activation of the CaMK pathway activates CaMKIV and promotes insulin synthesis in pancreatic β cells [23].

We investigated the mechanism that stimulates insulin gene transcription by focusing on the effects of changes in glucose concentration. Exposure of INS-1 cells to 11.2 mmol/l glucose increased insulin promoter activity, which in turn increased CaMKIV activity. The constitutively active form of CaMKIV was then transfected into pancreatic β cells. We checked the changes under these conditions and found that insulin promoter activity was upregulated. In addition, transfection of CaMKIV dominant-negative mutants into INS-1 cells markedly suppressed glucose-induced insulin promoter activity. These results indicate that the CaMK pathway may be essential in the transcriptional activation of insulin genes in response to changes in glucose concentration. In view of the above, it is necessary to examine markers associated with glucotoxicity in order to further develop the present study. In addition to apoptosis markers such as caspase and calpain, AGEs, reactive oxygen species, inflammatory markers and Endoplasmic Reticulum (ER) stress markers, which may serve as markers for the development of glucotoxicity, could be examined in more detail in relation to glucotoxicity and ABCA1. In addition, the relationship between ABCA1 and CaMKIV requires further investigation. We have previously reported on a direct relationship between ABCA1 and CaMKIV in hepatocytes [24]: the CaMKK / CaMKIV / PREB signalling pathway regulates ABCA1 expression in the liver and the GLP-1, Excendin-4 treatment enhanced ABCA1 expression via the CaMKIV pathway and suppressed cholesterol accumulation in hepatocytes.

In the present study, we showed that K-877 is involved in the activation of both CaMKIV and ABCA1. Based on these findings, it is likely that K-877 directly activates CaMKIV. On the other hand, as a further issue, it is very important to check how the loss of CaMKIV function affects the expression of ABCA1 and how it results in changes in GSIS. This study has several important clinical implications. Previous clinical trials have shown that K-877 has little effect on glucose intolerance. Araki, et al. [25] examined the efficacy of pemafibrate for 52 weeks in hypertriglyceridemic patients with concomitant type 2 diabetes. As a result of this report, pemafibrate improved the lipid profile associated with patients with type 2 diabetes, including hypertriglyceridaemia and hypo-HDL cholesterolaemia, while it did not significantly alter glucose metabolism. Based on these findings, the efficacy of pemafibrate should be carefully evaluated when used in clinical practice. On the other hand, K-877 has recently been reported to be effective in reducing postprandial hyperglycemia in mice [26]. The improvement in GSIS by K-877, which we clarified in this study, may be involved in the improvement of postprandial hyperglycemia. In conclusion, our results suggested that K-877 may affect insulin secretion by regulating ABCA1 expression in pancreatic β-cells in a glucotoxic state. This finding provides a new therapeutic target for glucotoxicity in diabetes mellitus.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author, K.F., upon reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Author Contributions

Conceptualization, K.F.., J.L., H.I. and K.M.; methodology, K.F., J.L., H.I., J.W., T.K., T.S., T.Y., S.S., and N.S.; validation, J.L., H.I., J.W., T.K., T.S., T.Y., and S.S.; investigation, K.F. and J.L.; resources, K.F., J.L., H.I., J.W., T.K., T.S., T.Y., and S.S.; data curation, K.F.; writing-original draft preparation, K.F. and K.M.; writing-review and editing, K.F. and K.M.; supervision, H.I. and K.M.; project administration, K.M.; funding acquisition, K.M., and K.F.. All authors have read and agreed to the published version of the manuscript.

Compliance with Ethical Standards

This article does not contain any studies with human or animal subjects performed by any of the authors.

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