DPP-4 Inhibition Ameliorates Pancreatic β-Cell Failure and Improves Glucose Tolerance in the Mouse Model of Wolfram Syndrome

Yasuhiro Tanji1, Suguru Yamaguchi1,2, Yasushi Ishigaki1, Hideki Katagiri1, Yoshitomo Oka1, Hisamitsu Ishihara2*

1Division of Molecular Metabolism and Diabetes, Tohoku University Graduate School of Medicine, Sendai, Japan
2Division of Diabetes and Metabolic Diseases, Nihon University School of Medicine, Tokyo, Japan
Email: *ishihara.hisamitsu@nihon-u.ac.jp

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Abstract

Wolfram syndrome, an autosomal recessive disorder associated with diabetes and optic atrophy, is caused by mutations in the WFS1 gene encoding wolframin, an endoplasmic reticulum membrane protein. Recent development of incretin-based drugs demonstrates promising outcomes for treatment of diabetes mellitus. The aim of this study is to evaluate whether dipeptidyl peptidase-4 inhibition is effective for treating endoplasmic reticulum stress-mediated β-cell failure and impaired glucose tolerance in WFS1-deficient mice (Wfs1−/−). Wfs1−/− mice were orally administrated with vildagliptin (50 mg/kg), a dipeptidyl peptidase-4 inhibitor, twice a day for 4 weeks. The pancreases of these mice were subjected to morphological and biochemical analyses and their glucose tolerance was studied. Electron microscopic studies revealed that vildagliptin reduced number of β-cell containing swollen endoplasmic reticulum in Wfs1−/− mice. Vildagliptin treatment increased pancreatic insulin content by 30% in Wfs1−/− mice. Oral and intraperitoneal glucose tolerance tests showed improved glucose tolerance in vildagliptin-treated Wfs1−/− mice with increased glucose responsiveness of insulin secretion as compared with vehicle-treated mutant mice. These effects by dipeptidyl peptidase-4 inhibition were partly prevented by glucagon-like peptide-1 receptor blockade. These findings provide evidence that activation of the incretin system by dipeptidyl peptidase-4 inhibition plays a protective role against β-cell failure in wolframin-deficiency. Our data suggest that diabetes in patients affected with Wolfram syndrome could be treated by incretin-based drugs. Furthermore, since WFS1 dysfunction could be involved in common forms of diabetes.

*Corresponding author.

type 2 diabetes mellitus, our results strengthen the mechanistic rational of using this drug for the disease.

**Keywords**

Diabetes, Incretin, Endoplasmic Reticulum Stress, WFS1, Wolframin

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### 1. Introduction

Wolfram syndrome is a rare autosomal recessive disorder characterized by juvenile-onset diabetes mellitus, optic atrophy, diabetes insipidus and sensorineural deafness [1]. This syndrome is caused by mutations in the WFS1 gene, which encodes wolframin, an endoplasmic reticulum (ER) resident membrane protein [2] [3]. Interestingly, recent studies indicated sequence variants in this gene confer risk of type 2 diabetes [4] [5]. We have generated Wfs1 knock-out mice which develop impaired glucose homeostasis due to progressive β-cell loss [6].

The β-cell failure in wolframin-deficiency was then revealed to result from increased ER stress [7].

ER stress is triggered when the amount of unfolded and misfolded proteins exceeds the folding capacity of the ER. To cope with this stress, cells activate intracellular signaling pathways, termed the unfolded protein response [8]. However, when ER stress extensively impairs ER homeostasis, the apoptotic pathways are triggered, leading to cell death. Recent studies revealed that one of the causes of β-cell loss in diabetes is ER stress-mediated apoptosis [9].

Incretin hormones, glucagon-like peptide-1(GLP-1) and glucose-dependent insulinotropic peptide (GIP), stimulate insulin secretion in a glucose-dependent manner. Activation of GLP-1 receptors [10]-[12] or GIP receptors [13] has also been shown to maintain and/or increase β-cell mass. In the blood stream, GLP-1 and GIP are immediately degraded by dipeptidyl peptidase 4 (DPP-4). To raise circulating levels of active incretins, DPP-4 inhibitors are clinically used as a novel strategy for treating diabetes. It has recently been shown that DPP-4 inhibitor vildagliptin ameliorates ER stress in β-cells of a mouse model of diabetes [14]. Here, we examined whether DPP-4 inhibition is beneficial for diabetes in Wolfram syndrome, using a mouse model of the disease.

### 2. Materials and Methods

#### 2.1. Animals

All animal experiments were approved by Tohoku University Institutional Animal Care and Use Committee. Wfs1−/− mice and their wild-type littermates had a C57BL/6 background and were described previously [6]. Only male mice were studied. All mice were housed one mouse per cage under a light/dark cycle of 12 hours. Either vildagliptin (Novartis pharma AG Switzerland, 50 mg/kg body weight) or vehicle (water) was orally administered twice a day (at 9:00 A.M. and 7:00 P.M.) at a volume of 10 mL/kg. In some experiments, Wfs1−/− mice were intraperitoneally injected with either exendin (9 - 39) (30 nmol/body weight) or saline 45 min before treatment with vildagliptin or vehicle.

#### 2.2. Physiological Studies

Food intake and body weight were measured twice a week. Blood samples were collected from the tail vein. Blood glucose was measured using a Glutest glucose sensor device (Sanwa Kagaku Kenkyusho, Nagoya, Japan) which monitors electric current generated through glucose oxidation to gluconic acid by glucose oxidase. Serum insulin levels were determined using an insulin ELISA kit (Morinaga Institute of Biological Science, Tokyo, Japan). For analysis of active GLP-1, mice were administered with 20% glucose solution (2 g/kg body weight), and 15 min later, mice were killed and blood was collected into tubes containing a DPP-4 inhibitor (Millipore Billerica, MA), and analyzed using an active GLP-1 (7 - 36) ELISA kit (Linco Research Inc., St Charles, MO). For an oral glucose tolerance test (OGTT) and intraperitoneal glucose tolerance test (IPGTT), animals after 16 hour-fast were administered with 20% glucose solution (2 g/kg body weight). Laboratory technicians have performed analyses kept blind to treatment allocation by numbering the sample tubes serially. Glucose tolerance-tests have been performed by technicians with mouse genotypes being kept blind.
2.3. Pancreas Insulin Content

Pancreases were excised and minced by scissors in acid ethanol, and left at −20°C for 48 hours, with sonication every 24 hours. Insulin concentrations in the acid ethanol were determined with an insulin ELISA Kit (Morinaga).

2.4. Transmission Electron Microscopy

The samples of pancreases were fixed with 2% glutaraldehyde plus 2% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) at 4°C, and subsequently post-fixed with 2% osmium tetroxide in 0.1 M PB at 4°C for 2 hours. Then, the specimens were dehydrated in agraded ethanol, replaced in propyleneoxide, and embedded in the epoxy resin. Ultrathin sections were obtained by ultramicrotomy technique. Ultrathin sections stained with uranyl acetate at 60°C for 20 minutes and modified Sato’s lead solution for 5 minutes were submitted to Transmission Electron Microscope observation (JEM-1200EX, JEOL). Electron micrographs of β-cells were randomly selected and examined in a masked manner. A β-cell with significantly swollen ER visible at 6600 × magnification (at least 50 nm in width) is counted for a positive β-cell with swollen ER.

2.5. Statistical Analysis

Data are presented as means ± SE. Differences between groups were assessed by Student’s t test. P < 0.05 was considered significant.

3. Results

3.1. A Single Dose of Vildagliptin Enhanced β-Cell Responsiveness in Wfs1−/− Mice

In order to determine whether acute effects of vildagliptin are preserved in WFS1-deficient mice, an oral glucose tolerance test (OGTT) was performed after a single administration of the drug into male wild-type (WT) and Wfs1−/− mice. We used mice at 5 weeks of age when Wfs1−/− mice on the C57BL/6 background show no apparent increases in blood glucose levels [6]. The mice were fasted overnight, and vildagliptin (50 mg/kg) was given through gastric tubes. Forty five minutes after vildagliptin administration, an OGTT was started. Plasma levels of active GLP-1, one of main DPP-4 targets, measured 15 minutes after glucose loading, were increased in Wfs1−/− mice as in WT mice by a single dose of vildagliptin (Figure 1(a)). Vildagliptin reduced glycemic excursion in both WT and Wfs1−/− mice (Figure 1(b)). The plasma insulin over glucose levels at 15 min after glucose load were significantly elevated, indicating that β-cell responsiveness was increased by the prior treatment with vildagliptin in both strains (Figure 1(c)). These data suggested that principle mechanisms of a DPP-4 inhibitor are preserved in the wolframin-deficient mice.

3.2. Four-Week Treatment with Vildagliptin Ameliorated β-Cell Failure in Wfs1−/− Mice

To investigate effects of continuous administration of vildagliptin on β-cell failure and impaired glucose tolerance in Wfs1−/− mice, vildagliptin (50 mg/kg) was orally administrated twice a day for 4 weeks, starting at 5 weeks of age. There was no difference in body weight throughout the study between vehicle- and vildagliptin-administrated mice of both strains (data not shown). The average daily food intake also showed no differences among four groups (data not shown). Similar blood glucose levels measured in non-fasted states were observed among four groups during 4-week administration period (data not shown).

Since we showed that β-cell failure in Wfs1−/− mice results from increased ER stress [7] and recent data indicated DPP-4 inhibition ameliorates ER stress in the β-cell [14], we had expected that β-cell ER stress might be improved in WFS1-deficient mice treated with vildagliptin. To investigate this possibility, ER morphology was analyzed by electron microscopic method in these mice. Consistent with the fact that wolframin-deficient β-cells are suffered from ER stress, β-cells with swollen ER, a typical phenotype of ER-stressed β-cells [15], was frequently observed in Wfs1−/− mice (Figure 2(a)). Vildagliptin treatment for 4 weeks resulted in reduction in cell numbers with swollen ER, indicating that enhancement of incretin action reduced ER stress in wolframin-deficient β-cells.

After 4-week administration of vildagliptin, we evaluated pancreatic insulin content of these mice. Pancreatic insulin content of vehicle-treated Wfs1−/− mice at 9 weeks of age was decreased to 47 ± 5% (p < 0.01)
Figure 1. Oral glucose tolerance tests (2 g/kg body weight) were performed 45 min after a single treatment of vildagliptin vehicle, in male WT mice and \( Wfs1^{-/-} \) mice (KO) at 5 weeks of age. (a) Active GLP-1 levels were measured at 15 min after glucose load. \( * p < 0.05 \). (b) Blood glucose levels were lower in vildagliptin-treated mice. \( * p < 0.05 \) between vildagliptin- and vehicle-treated WT mice. \( \dagger p < 0.05 \) between vildagliptin- and vehicle-treated \( Wfs1^{-/-} \) mice. \( \dagger\dagger p < 0.01 \) between vildagliptin- and vehicle-treated \( Wfs1^{-/-} \) mice. (c) Insulin secretory response was calculated by dividing changes of plasma insulin levels during 15 min by changes of blood glucose. \( * p < 0.05 \). Data are presented as means ± SE, \( n = 7 - 12 \) mice.

Figure 2. (a) Ultrastrucual analysis was performed on pancreatic sections from vehicle-treated WT mice (\( n = 1 \)), vildagliptin-treated WT mice (\( n = 1 \)), vehicle-treated \( Wfs1^{-/-} \) mice (KO, \( n = 2 \)), and vildagliptin-treated \( Wfs1^{-/-} \) mice (\( n = 2 \)). The bar graph represents the percentage of \( \beta \)-cells with swollen ER of each mouse, and counted numbers of \( \beta \)-cells were shown in the bars. Representative images of electron micrographs are shown. N: nucleus, SG: secretory granule, M: mitochondria, ER: endoplasmic reticulum, scale bars: 500 nm. (b) Insulin content extracted from whole pancreata. Data are presented as means ± SE, \( n = 7 - 9 \) mice. \( * p < 0.05 \).

as compared with that of WT mice (Figure 2(b)), consistent with our previous report [6]. Vildagliptin treatment increased pancreatic insulin content to 133 ± 12% (\( p < 0.05 \)) in \( Wfs1^{-/-} \) mice, while vildagliptin unaltered the
parameter in WT mice (Figure 2(b)).

3.3. Four-Week Treatment with Vildagliptin Improved Glucose Tolerance in Wfs1−/− Mice

In order to determine effects on glucose tolerance of long-term DPP-4 inhibition, we planned to conduct an intraperitoneal glucose tolerance test (IPGTT). IPGTT was chosen because unaltered glucose tolerance during IPGTT in the GLP-1 and GIP receptors double knockout mice indicated that acute incretin activation was not directly involved when glucose was administered intraperitoneally [16], allowing separation of long-term effects on glucose tolerance. Nonetheless, before conducting IPGTT, OGTT was performed after 3-week treatment with vildagliptin. Overnight-fasted mice were challenged with glucose 24 h after the last administration of vildagliptin. Blood glucose excursions were lower in WT mice treated with vildagliptin for 3 weeks than in vehicle-treated controls (Figure 3(a)). As reported previously [6], Wfs1−/− mice started to develop impaired glucose tolerance at 8 weeks of age, and their glycemic levels after oral glucose challenge were reduced by vildagliptin treatment.

![Graphs showing glucose tolerance test results](image)

**Figure 3.** (a) (b) Oral glucose tolerance tests (2 g/kg body weight) were performed in WT and Wfs1−/− mice (KO) at 8 weeks of age with or without 4-week vildagliptin treatment. (a) Blood glucose levels were lower in vildagliptin-treated mice. *p < 0.05 between vildagliptin- and vehicle-treated WT mice. †p < 0.05 between vildagliptin- and vehicle-treated Wfs1−/− mice. (b) Insulin secretory response was calculated by dividing changes of plasma insulin levels during 15 min by changes of blood glucose. †p < 0.05. ‡p < 0.01. (c) (d) Intraperitoneal glucose tolerance tests (2 g/kg body weight) were performed in WT and Wfs1−/− mice at 9 weeks of age with or without 4-week vildagliptin treatment. (c) Blood glucose levels were lower in vildagliptin-treated Wfs1−/− mice. †p < 0.05 between vildagliptin- and vehicle-treated Wfs1−/− mice. (d) Insulin secretory response was calculated as in (b). *p < 0.05. Data are presented as means ± SE, n = 5 - 7 mice.
(Figure 3(a)). Plasma insulin over glucose ratios determined 15 min after glucose load were higher in vildagliptin- than in vehicle-treated mice in both strains (Figure 3(b)).

WT and Wfs1−/− mice at 9 weeks of age were then subjected to an IPGTT 24 h after the last administration of vildagliptin. There was no difference in glucose excursion (Figure 3(c)) and insulin secretion (Figure 3(d)) between vehicle- and vildagliptin-treated WT mice. Interestingly, vildagliptin-treated WFS1-deficient mice showed improved glucose tolerance as compared with vehicle-treated Wfs1−/− mice (Figure 3(c)), which was accompanied with improved glucose responsiveness in insulin secretion (Figure 3(d)). There seems to be no effects on whole body insulin sensitivity of vildagliptin treatment, since insulin (0.75 U/kg) tolerance test showed no differences in glucose lowering among four groups of mice at 9 weeks of age (data not shown).

3.4. The β-Cell Protective Effect of Vildagliptin Was Partly Blocked by Exendin (9 - 39)

The effects of DPP-4 inhibitors on glucose homeostasis are complicated, since a number of physiologically active substances including various hormones and cytokines, are regulated by DPP-4. In order to examine that the effects obtained by long-term treatment with vildagliptin were attributed to enhanced action of GLP-1, we conducted experiments using a GLP-1 receptor antagonist exendin (9 - 39). Exendin (9 - 39) (30 nmol/kg body weight) was intraperitoneally administrated twice a day in combination with vildagliptin or vehicle to Wfs1−/− mice for 4 weeks.

Pancreatic insulin content of these mice was analyzed. Similar to data shown in Figure 2(b), insulin content of vildagliptin-treated Wfs1−/− mouse pancreases was increased to 128 ± 10% (p < 0.05) as compared with that of vehicle-treated ones (Figure 4(a)). However, exendin (9 - 39) treatment blocked vildagliptin-induced increases in pancreatic insulin content (Figure 4(a)). These results demonstrate that protective effects of vildagliptin on Wfs1−/− mice were, at least partly, through active GLP-1.

We also analyzed glucose tolerance of these mice after 4-week treatment. The overnight-fasted mice at 9 weeks of age were subjected to an IPGTT 24 h after the last administration of the drugs. Although blood glucose levels were significantly lower in vildagliptin- than in vehicle-treated mice, there were no differences between vildagliptin plus exendin (9 - 39)-treated and vehicle-treated Wfs1−/− mice (Figure 4(b)). We also observed that vildagliptin-augmented plasma insulin over glucose ratios were reduced by exendin (9 - 39), although the difference between vildagliptin-induced increases in the ratios was not statistically significant between with and without exendin (9 - 39) co-treatment (Figure 4(c)).
4. Discussion

The present study showed that DPP-4 inhibition ameliorates β-cell failure and improves glucose tolerance in wolframin-deficient mice. Our data might be suggestive for the treatment strategy of diabetes in Wolfram syndrome patients.

We observed that vildagliptin treatment reduced β-cell numbers with swollen ER, a typical phenotype of ER stressed cells. The result suggested that vildagliptin reduced ER stress per se or made β-cells resistant to ER stress, leading to improvement of cellular homeostasis of WFS1-deficient β-cells. ER stress conditions could be analyzed by changes in ER stress sensor or marker protein expressions. Previous studies have shown that expressions of ER stress markers, ATF4 and GRP78, were increased by GLP-1-receptor agonist, exendin-4 [17][18]. Increased expression of ATF4 and GRP78 is also observed in cells undergoing apoptosis caused by ER-stress inducers. Therefore, when one observed ATF4 and/or GPR78 induction in cells, it is difficult for one to judge or interpret whether cells are undergoing apoptosis or recovering from ER stress conditions. This is the reason why we evaluated ER stress conditions morphologically, as in a report by Scheuner et al. [15].

Theoretically, since effects of DPP-4 inhibitors and of incretin hormones are diverse, it was possible that improvement of glucose tolerance in Wfs1−/− mice was due to vildagliptin-activated incretin actions outside β-cells. Nonetheless, we believe that the major contributor for improved glucose tolerance was restoration of β-cell function, because the primary cause of diabetes is β-cell dysfunction in Wfs1−/− mice. In addition, an interesting observation in our study was that glucose tolerance was improved when glucose was challenged through intraperitoneally in Wfs1−/− mice. Since incretin system might not be involved in glucose tolerance after intraperitoneal glucose challenge, improved glucose responsiveness of insulin secretion from Wfs1−/− mice treated with vildagliptin indicated restored β-cell function per se, which might include enhanced responsiveness of individual β-cells and/or increased β-cell mass. Increases in pancreatic insulin content in Wfs1−/− mice treated with vildagliptin (Figure 2(b)) supported the latter mechanism.

In rodent diabetic models, GLP-1 receptor agonists and DPP-4 inhibitors have been reported to have beneficial effects on β-cell mass through stimulating proliferation, preventing cell death, or promoting neogenesis [14][19]-[22]. An interesting observation has been reported in β-cell specific C/EBPB transgenic mice suffering from β-cell loss caused by increased ER stress [23]. Importantly, vildagliptin treatment has been shown to ameliorate ER stress through C/EBPB degradation in the mutant mouse β-cells. Since function of WFS1 protein remains elusive, mechanisms by which vildagliptin protects β-cell from ER stress in Wfs1−/− mice is unknown and should be solved in the near future.

The present data suggest incretin-based therapy as a potential therapeutic option for Wolfram syndrome. Since recent studies have revealed neuroprotective effects of GLP-1 [24], it will be quite intriguing to see whether symptoms outside β-cells of Wolfram syndrome, such as diabetes insipidus and optic atrophy, can be suppressed by incretin-based drugs. Very recently, wolframin-deficient β-cells have been generated from human induced pluripotent stem (iPS) cells derived from subjects with Wolfram syndrome [25]. Wolfram β-cells showed increased levels of ER stress markers and reduced insulin secretion. Remarkably, a chemical chaperon has been reported to upregulate insulin secretion. Another report generated neural progenitor cells from iPS cells of Wolfram syndrome patients and a calcium-dependent protease has been shown to prevent cell death in these mutants [26]. These studies contribute to our understanding and development of therapeutic strategies not only for Wolfram syndrome but also for type 2 diabetes mellitus, since WFS1 abnormalities are linked to common forms of type 2 diabetes mellitus.

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Conflict of Interest

HI has received lecture fees from Asteras, AstraZeneca, Boehringer Ingelheim, Daiichi Sankyo Inc., Eli Lilly and Company, Kowa Pharmaceutical Co., MSD, Novartis Pharmaceuticals, Novo Nordisk Pharma, Ono Pharmaceutical Co., Mitsubishi Tanabe Pharma, and Sanofi, and research funds from Asteras, Boehringer Ingelheim.
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