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Vascular protection of DPP-4 inhibitors in retinal endothelial cells in in vitro culture

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ABSTRACT

People with diabetes are at high risk of developing diabetes-related eye disease, termed as diabetic retinopathy, due damage being caused to the blood vessels in the retina. An efficient medical treatment to reduce diabetic retinopathy can improve the quality of life for diabetes patients. In our study, we show that linagliptin, a commercially available DPP-4 inhibitor, plays a protective role in retinal vascular endothelial cells. The presence of linagliptin protects retinal endothelial cells against TNF-α-induced cytotoxicity and enhances their viability. Linagliptin treatment suppresses TNF-α-induced production of reactive oxygen species and improves mitochondrial membrane potential. Moreover, linagliptin suppresses TNF-α-induced production of pro-inflammatory and pro-adhesive vascular cytokines including IL-6, IL-8, ICAM-1, and VCAM-1. The presence of linagliptin in cell media can reduce the number of THP-1 cells that adhere to retina endothelial cells. Mechanistically, linagliptin potently suppresses TNF-α-induced accumulation of NF-κB nuclear protein p65 and activation of NF-κB promoter. Our data indicate that linagliptin is an anti-inflammatory diabetic agent, with the potential to be applied as a treatment for diabetic retinopathy.

1. Introduction

Diabetes mellitus is a serious worldwide health problem. The higher prevalence of diabetes seen in recent decades increases the risk of serious diabetes complications. Diabetic retinopathy is one of the major complications of diabetes and the main cause of diabetes-related blindness [1]. It has been estimated that approximately one third of diabetes patients have signs of retinopathy and many of them have vision-threatening risk factors [2].

The retina is a specialized tissue that converts visible light into the neuronal signals which are perceived by the brain. The retina's unique vascular system provides nutrients and oxygen to the inner and outer retina, the integrity of which is essential for sensing light. The retinal vascular structure has features similar to the blood brain barrier and is highly sensitive to the microenvironment. Upon prolonged exposure to chronic hyperglycemia conditions, retinal endothelial cells undergo a range of unique structural changes, such as altered permeability, hyperproliferation of endothelial cells and edema, and abnormal vascularization of the retina, resulting in loss of vision. Extensive studies have demonstrated that oxidative stress and inflammation are linked to one another and act as significant drivers of these diabetic complications. Recent research has been focused on specific mechanism-based strategies to target both oxidative stress and inflammatory pathways, thereby improving the complication burden of diabetes patients, including retinopathy [3]. Often the early stages of diabetic retinopathy have no visual symptoms, so early detection and treatment is of utmost importance in preventing significant vision loss from diabetic retinopathy. It has been proposed that an efficient early medical treatment would be significant in preventing further vision loss due to diabetic retinopathy.

Gliptins have become a part of various therapeutic regimens to treat type 2 diabetics in recent decades. Gliptins were developed to lower the blood glucose in type 2 diabetes patients and have been shown to be effective [4,5]. More than a dozen gliptins have been developed for the treatment of T2DM, including the most commonly used alogliptin, linagliptin, saxagliptin, sitagliptin, and vildagliptin [6]. In this study, we report that linagliptin, a commercially available DPP-4 inhibitor, actually exerts a protective effect in cultured retinal vascular endothelial cells.

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2. Materials and methods

2.1. Cell culture, treatment, and monocyte adhesion experiment

Primary human retina endothelial cells (RECs, ACBRI 181) were purchased from Cell Systems (Kirkland, WA), and human monocytes cells line THP1 was from ATCC (Manassas, VA). Linagliptin was from Sigma-Aldrich and dissolved in PBS. The RECs were grown in 10% serum growth media supplemented with all the growth factors and used in low passage numbers (< 10). The THP1 cells were maintained in 10% fetal serum containing in DMEM media. All the cell experiments were housed in 5% (v/v) CO_2/95% (v/v) nitrogen incubator at 37 °C. For TNF-α and linagliptin treatment experiments, 10 ng/ml TNF-α-containing media was added to confluent REC cells in the presence or absence of linagliptin (50 and 100 nM) for 24–72 h [7]. For monocyte-endothelial cell adhesion experiments, the assay was performed by adding 5×10^5 THP1 cells to 1×10^5 confluent REC cells for 2 h, then adhesive THP1 cells were washed and stained by the cell-permeant dye calcein-AM with green fluorescence. The green fluorescence-labeled cells were counted and normalized to total REC cell numbers.

2.2. MTT and LDH assay

The viability of RECs was measured by MTT assay. Briefly, cells were incubated for 4 h with 0.8 mg/ml of MTT in serum-free medium, followed by the addition of DMSO. The stabilized cell-MTT reaction mixture was transferred into 96-well plates and absorbance was recorded at 560 nm using a microplates spectrophotometer system. LDH cytotoxicity was assessed by leakage of lactate dehydrogenase (LDH) into the culture medium. The culture medium was collected to obtain the cell-free supernatants from the different conditions. The activity of LDH in the medium was determined using a commercially available kit from Thermo Fisher Scientific, USA. The data were analyzed and are presented as a percentage of the control values.

2.3. Quantitative real-time PCR

The total RNAs from RECs were extracted with RNeasy Micro Kit from Qiagen (Hilden, Germany) in accordance with the manufacturer's manual. The RNA concentrations were quantified by a Nanodrop spectrophotometer from Cole-Parmer (Chicago, IL). A total of 1 μg of RNA was used to synthesize the cDNA using iScript™ Reverse Transcription Supermix for RT-qPCR from Invitrogen (Carlsbad, CA). SYBR-based real-time PCR experiments were performed to detect the total transcripts of mRNA of VCAM-1, ICAM-1, IL-6, and IL-8 by ABI 7500 platform.

2.4. Western blot analysis

RECs under different conditions were lysed by RIPA buffer with a protease inhibitor. A total of 20 μg cell lysates were loaded into 4–20% precasted PAGE gel to separate the proteins according to size. The separated protein mix was transferred onto PVDF membranes to detect the corresponding protein levels by specific antibodies. The antibodies used were: ICAM-1, VCAM-1, p65, lamin B, and β-actin.

2.5. Nuclear extracts

The nuclear extracts of RECs were extracted using a kit from Thermo Fisher Scientific in accordance with the manufacturer's instructions. The nuclear protein lamin B was used as a quality control. The nuclear fraction of p65 protein level was examined to determine NF-κB activation.

2.6. ELISA

To measure the secreted levels of IL-6 and IL-8 cytokines under the different conditions, the REC culture media was collected for analyses. Two ELISA kits were purchased from R&D Systems. The experiments were performed by following the manufacturer's instructions. The data were collected using 96-well plate reader spectrometry. The absolute values were obtained from a standardized 4-PL curve. The relative levels of IL-6 and IL-8 are presented as normalized to total protein amounts in each condition.

2.7. ROS and 4-HNE assay

Cellular reactive oxygen (ROS) production was measured by quick staining the cells under the different conditions with 2′,7′–dichlorofluorescin diacetate dye (DCFH-DA). The fluorescent image density was captured using a fluorescence microscope. The fluorescent density of images was quantified using Image J software. 4-HNE (4-hydroxynonenal) is the major byproduct of lipid peroxidation during oxidative stress. We measured 4-HNE levels using an immunofluorescence method. In brief, the cells were grown on coated slides and treated under the desired conditions. The cells were then fixed by 4% paraformaldehyde and permeabilized by 0.1% Triton-X 100. Cells were stained with anti-HNE antibody followed by incubation with Alexa 594 conjugated secondary antibody to visualize the image. The stained images were quantified by fluorescence density using software Image J.

2.8. Mitochondrial membrane potential assay

Mitochondrial membrane potential (MMP) assay was based on measurement of TMRM staining (tetramethyl rhodamine methyl and ethyl esters). Based on the concentration of TMRM accumulated in the mitochondria, the aggregated fluorescent counts were measured to determine the depolarization of mitochondria and cell health status. We purchased the TMRM kit from_Bio-Rad, USA and MMP activity was measured with fluorescent density and quantified to present the data.

2.9. NF-κB promoter assay

The 3× NF-κB binding site-containing luciferase vector was purchased from Thermo Fisher Scientific. Cells were co-transfected with NF-κB promoter and a firefly luciferase promoter by Lipofectamine 2000 reagents from Invitrogen (Carlsbad, CA). 24 h post-transfection, cells were switched to 30 mM glucose media in the presence or absence of linagliptin at the concentrations of 5 and 10 μM for an additional 48 h. The total cell lysates were collected to measure the dual luciferase activity of renila and firefly luciferase. The relative luciferase was calculated by normalizing the activity of firefly luciferase to renilla luciferase activity.

3. Results

3.1. Linagliptin

Linagliptin has a xanthine-based structure (Fig. 1), and its molecular formula is $C_{25}H_{28}N_8O_2$. Like other gliptins, linagliptin is targeted to inhibit dipeptidyl-peptidase-4 (DPP-4). In this study, we investigated the possible vascular benefit of linagliptin in vascular endothelial cells.

3.2. Linagliptin ameliorates TNF-α-induced reduced cell viability and LDH release

Firstly, we tested the effects of co-treatment with linagliptin and TNF-α on retina endothelial cells by MTT assay. Prolonged cytokine treatment is known to induce endothelial death. After RECs were treated with 10 ng/ml TNF-α for two days, only about 50% RECs were

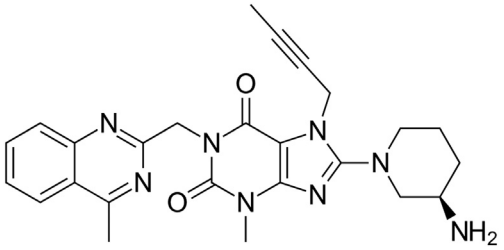

Fig. 1. Molecular structure of linagliptin.

viable. However, the presence of 50 and 100 nM linagliptin increased overall cell survival, with 65% and 80% RECs being viable under these two concentrations of linagliptin, respectively (Fig. 2A). At the same time, we tested the release of LDH resulting from above experiment. The same TNF-α treatment caused 5-fold higher release of LDH, while the addition of 50 and 100 nM linagliptin significantly suppressed LDH release, with only 3.5- and 2.5-fold release of LDH under these two concentrations of linagliptin, respectively (Fig. 2B).

3.3. Linagliptin suppresses TNF-α-induced generation of reactive oxygen species (ROS)

Endothelial cells have been shown to produce ROS in response to cytokine stimulation. We tested the effects of linagliptin on ROS production. Compared with non-treated cells, RECs produced 4-fold higher ROS on average under TNF-α treatment, while the addition of 50 and 100 nM linagliptin triggered only 2- and 1.5-fold higher production of ROS, respectively (Fig. 3A). We then tested the consequences of increased levels of ROS-lipid peroxidation product-4-hydroxynonenal (4-HNE). Compared to non-treatment cells, TNF-α induced 4-fold higher 4-HNE production on average. However, the addition of 50 and 100 nM linagliptin caused only 2- and 1.7-fold higher 4-HNE production, respectively (Fig. 3B). These data indicate that linagliptin could suppress cytokine-induced ROS production in RECs.

3.4. Linagliptin protects against TNF-α-induced mitochondrial dysfunction

Endothelial mitochondria are mainly responsible for generation of reactive oxygen species (ROS). Thus, we reasoned that the suppression of ROS production by linagliptin could be due to its protection of endothelial mitochondria under oxidative stress conditions. To test this hypothesis, we measured mitochondrial membrane potential in RECs. Compared to non-treatment cells, TNF-α treatment caused a 60% reduction in MMP, while the presence of 50 and 100 nM linagliptin resulted in only 40% and 20% reduction in MMP (Fig. 4), suggesting that linagliptin could protect RECs from mitochondrial dysfunction and oxidative stress.

3.5. Linagliptin inhibits TNF-α-induced production of inflammatory and vascular adhesion cytokines

Next, we tested the effect of linagliptin on cytokine-induced inflammatory responses. We measured expression of the two major pro-inflammatory cytokines IL-6 and IL-8 upon the exposure to TNF-α with or without linagliptin. Compared to non-treatment control cells, treatment of RECs by TNF-α resulted in an approximate 5.5-fold rise in IL-6 mRNA, while the addition of 50 and 100 nM linagliptin led to only approximate 3.5- and 2.5-fold higher IL-6 mRNA, respectively (Fig. 5A). Similarly, TNF-α treatment resulted in approximately 5-fold higher expression of IL-8, while the addition of the two doses of linagliptin resulted in only approximate 3- and 2-fold higher expression of IL-8 mRNA, respectively (Fig. 5A). We confirmed this inhibitory role of linagliptin by measuring cytokine secretion in the media of cultured cells. Compared to non-treated REC cells, the same TNF-α treatment condition resulted in approximately 3.5-fold higher IL-6 in the media, while the addition of 50 and 100 nM linagliptin resulted in only approximately 2.2- and 1.8-fold higher IL-6 production, respectively (Fig. 5B). Additionally, TNF-α resulted in approximately 3 fold higher IL-8 in the cultured media, while the addition of the two doses of linagliptin resulted in only approximately 2- and 1.5-fold higher IL-8 production, respectively (Fig. 5B).

It is unknown whether the inhibitory effects of linagliptin on inflammation and ROS generation occur via inhibition of DPP-4 activity. Therefore, we investigated the effects of another DPP-4 inhibitor sitagliptin on secretion of the cytokines IL-6 and IL-8, as well as ROS generation. As expected, the DCFH-DA results shown in Supplementary Fig. 1A indicate that treatment with sitagliptin reduced TNF-α-induced production of ROS in a dose-dependent manner. Correspondingly, the ELISA results shown in Supplementary Fig. 1B demonstrate that treatment with sitagliptin inhibited TNF-α-induced secretion of IL-6 and IL-8 in a dose-dependent manner. These results suggest that the inhibitory effects of linagliptin on inflammation and ROS generation might be dependent on the suppression of DPP-4 activity.

We then tested if linagliptin exerts a similar inhibitory effect on the production of vascular adhesion molecules. Indeed, our results indicate that linagliptin suppressed expression of the two vascular adhesion molecules ICAM-1 and VCAM-1 even more potently. Compared to non-treated cells, TNF-α treatment resulted in approximately 9-fold higher

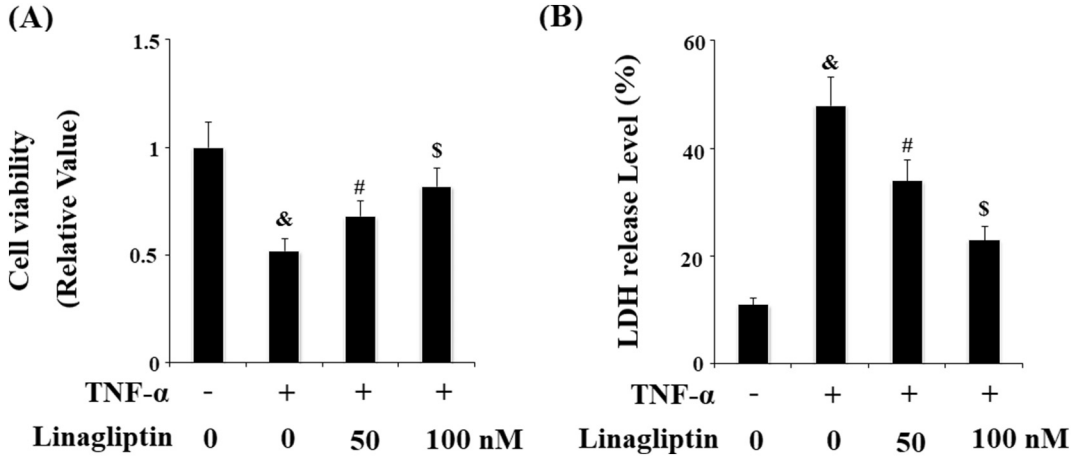

Fig. 2. Treatment with linagliptin ameliorates the TNF-α-induced reduction of cell viability and LDH release. Human primary retinal endothelial cells (RECs) were treated with TNF-α (10 ng/ml) in the absence or presence of linagliptin (50 nM and 100 nM) for 48 h. (A). Cell viability was determined by MTT assay; (B). LDH release was determined by a commercial kit (&, #, $, $P < 0.01$ vs. previous column group).

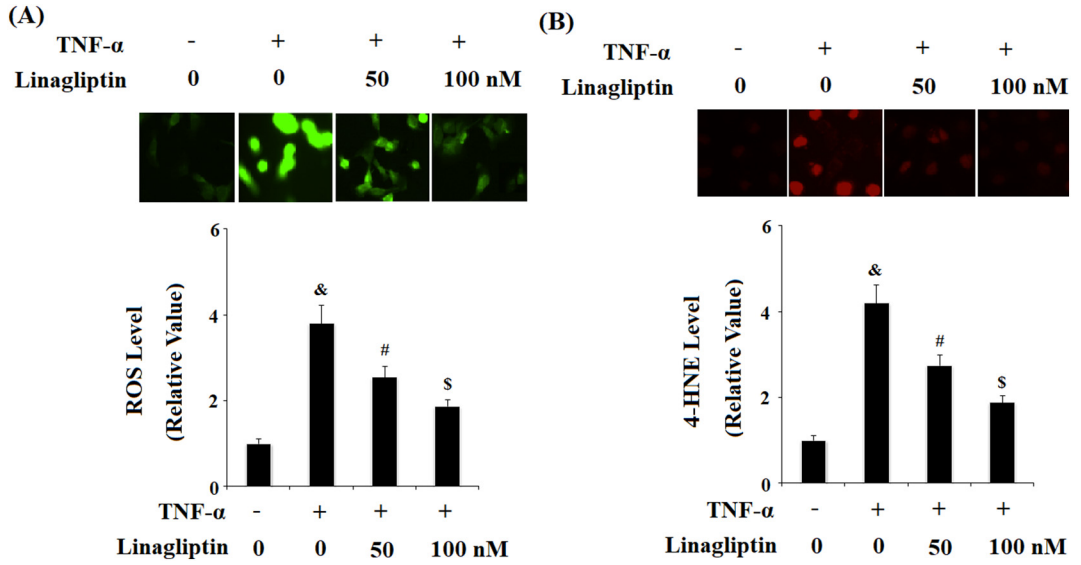

Fig. 3. Treatment with linagliptin suppresses TNF-α-induced generation of reactive oxygen species (ROS) and byproduct 4-hydroxynonenal (4-HNE). Human primary retinal endothelial cells (RECs) were treated with TNF-α (10 ng/ml) in the absence or presence of linagliptin (50 nM and 100 nM) for 24 h. (A). ROS was determined by the DCFH-DA method; (B). 4-HNE was determined by immunostaining (&, #, $, $P < 0.01$ vs. previous column group).

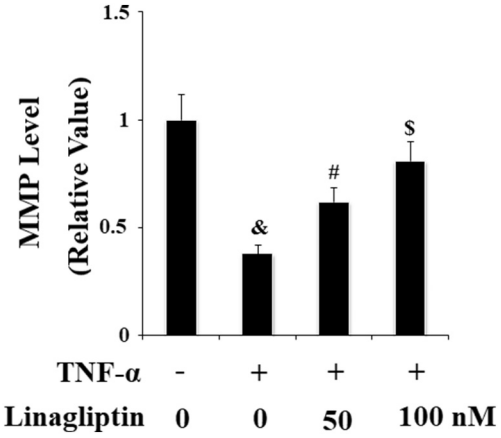

Fig. 4. Linagliptin improves the TNF-α-induced reduction in mitochondrial membrane potential (MMP). Human primary retinal endothelial cells (RECs) were treated with TNF-α (10 ng/ml) in the absence or presence of linagliptin (50 nM and 100 nM) for 24 h. Intracellular levels of MMP were determined by tetramethylrhodamine, methyl ester (TMRM) (&, #, $, $P < 0.01$ vs. previous column group).

ICAM-1 mRNA expression, while ICAM-1 expression was only approximately 5- and 3-fold higher in the presence of 50 and 100 nM linagliptin, respectively (Fig. 6A). Similarly, the same TNF-α condition resulted in approximately 10-fold higher VCAM-1 expression, while the presence of the two doses of linagliptin gave rise to only approximate 4- and 2-fold higher VCAM-1 mRNA. Again, we confirmed this inhibitory role of linagliptin by measuring the protein levels of these two molecules. Under basal non-treated conditions, ICAM-1 protein is barely detectable, while TNF-α treatment induced a large amount of ICAM-1 protein accumulation, but the addition of 50 and 100 nM linagliptin resulted in approximate 20% and 45% reductions in this protein (Fig. 6B). Similarly, VCAM-1 protein expression is non-detectable at the basal level, while TNF-α induced a large amount of VCAM-1 protein, but the two doses of linagliptin resulted in approximate 20% and 65% reductions in VCAM-1 (Fig. 6B).

3.6. Linagliptin inhibits TNF-α-induced adhesion of monocytes to retinal endothelial cells

Based on the inhibitory role of linagliptin on pro-inflammatory and pro-adhesive vascular adhesion cytokines, we tested whether linagliptin could suppress adhesion of monocytes to endothelial cells. We did the

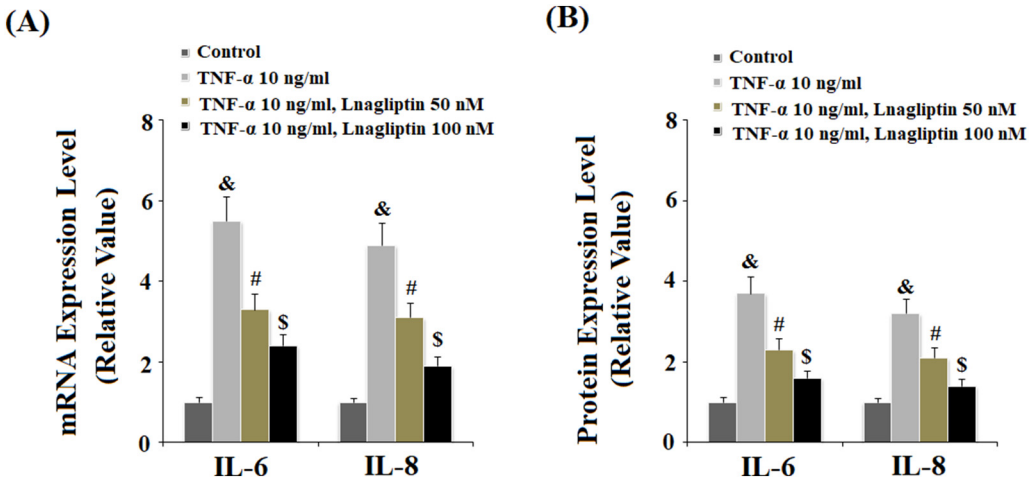

Fig. 5. Linagliptin inhibits TNF-α-induced generation of the pro-inflammatory cytokines IL-6 and IL-8. Human primary retinal endothelial cells (RECs) were treated with TNF-α (10 ng/ml) in the absence or presence of linagliptin (50 nM and 100 nM) for 24 h. (A). mRNA expression of IL-6 and IL-8; (B). Secretions of IL-6 and IL-8 were determined by ELISA (&, #, $, $P < 0.01$ vs. previous column group).

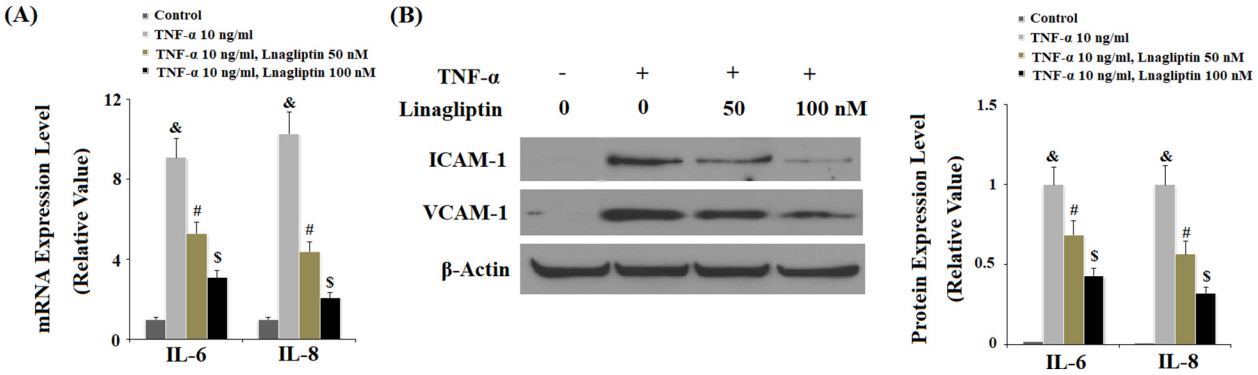

Fig. 6. Linagliptin inhibited TNF-α-induced expression of ICAM-1 and VCAM-1. Human primary retinal endothelial cells (RECs) were treated with TNF-α (10 ng/ml) in the absence or presence of linagliptin (50 nM and 100 nM) for 48 h. (A). mRNA expressions of ICAM-1 and VCAM-1 measured by real-time PCR; (B). Protein expressions of ICAM-1 and VCAM-1 measured by western blot (&, #, $, $P < 0.01$ vs. previous column group).

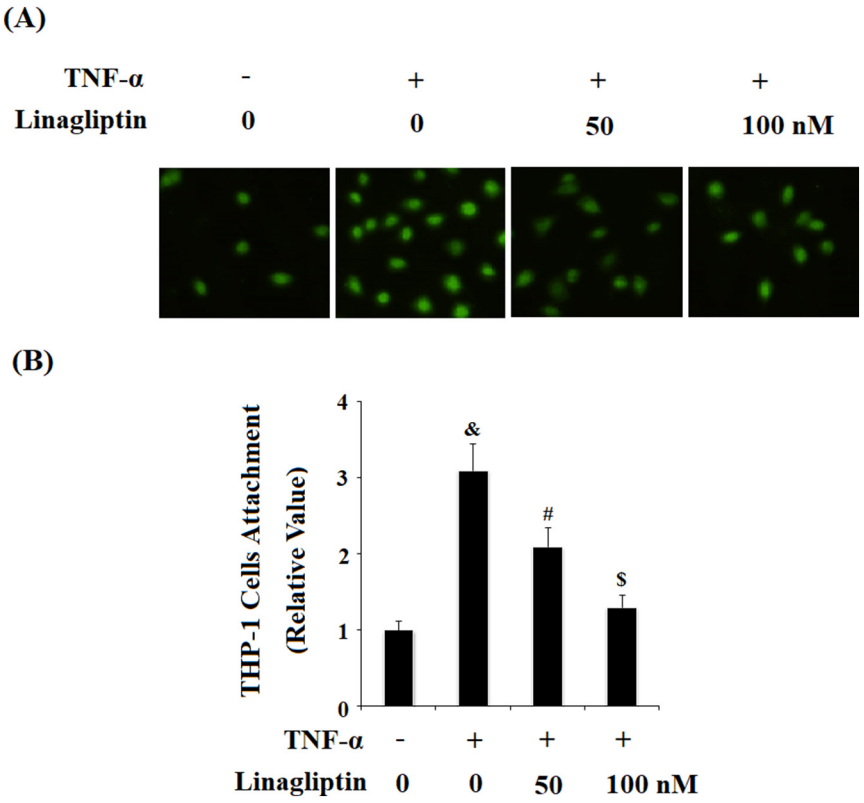

Fig. 7. Linagliptin inhibits TNF-α-induced THP-1 adhesion to retinal endothelial cells (RECs). Human primary retinal endothelial cells (RECs) were treated with TNF-α (10 ng/ml) in the presence of linagliptin (50 nM and 100 nM) for 24 h. (A) Representative images of the attached THP-1 cells to human primary retinal endothelial cells; (B). Quantitative analysis of the attached THP-1 cells (&, #, $, $P < 0.01$ vs. previous column group).

experiment by measuring the number of THP-1 monocytes adhered to cultured RECs after co-culture for 2 h. Clearly, the presence of TNF-α resulted in approximately 3-fold higher adhesion of THP-1 cells, but the addition of 50 and 100 nM linagliptin resulted in only 2- and 1.3-fold higher adhesion of THP-1 cells (Fig. 7A and B). These data suggest that linagliptin may strongly suppress TNF-α-induced adhesion of immune cells to RECs.

3.7. Linagliptin inhibits TNF-α-induced activation of NF-κB

Finally, we explored the possible inhibitory mechanism of linagliptin in inflammatory responses. We tested the influence of Linagliptin on NF-κB activation and found that linagliptin possessed strong inhibition of NF-κB initiation. We measured the accumulation of nuclear protein p65 upon exposure to TNF-α and linagliptin. Under basal non-treated conditions, there is a low level of nuclear p65 protein, while TNF-α treatment resulted in approximately 3-fold higher accumulation of p65 in the nucleus. However, the addition of 50 and 100 nM linagliptin led to only approximate 2.1- and 1.4-fold higher p65 accumulation, respectively. It should be noted that TNF-α-induced nuclear translocation of p65 was completely prevented by transfection with the IκBα dominant negative mutation (IκBα DN) (Fig. 8A). We then measured the activity of transfected NF-κB promoter. Without cytokine stimulation, the transfected promoter had a very low level of luciferase activity. However, treatment with TNF-α resulted in approximately 27-fold higher promoter activity, but the addition of the two doses of linagliptin led to only 10- and 5-fold higher promoter activity, respectively. Consistently, the TNF-α-induced increase in luciferase activity was blocked by transfection with IκBα DN. Importantly, blockage of NF-κB activation by transfection with IκBα DN abolished TNF-α-induced attachment of THP-1 cells to RECs (Supplementary Fig. 2). These results suggest that the inhibitory effects of linagliptin against TNF-α-induced endothelial dysfunction might be mediated by suppression of NF-κB.

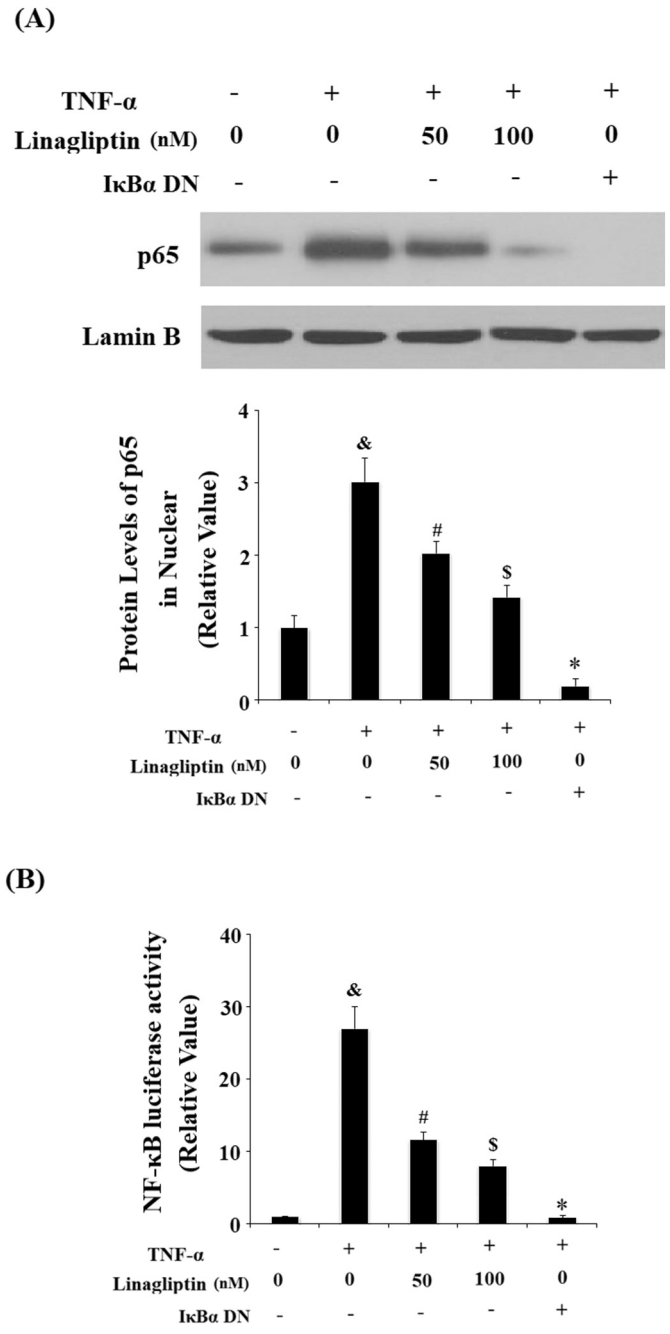

Fig. 8. Linagliptin suppresses TNF-α-induced activation of NF-κB. Human primary retinal endothelial cells (RECs) were treated with TNF-α (10 ng/ml) in the absence or presence of linagliptin (50 nM and 100 nM) for 24 h. RECs transfected with IκBα dominant negative mutation (IκBα DN) were used as a "loss of function" experiment. (A). Nuclear levels of p65; (B). NF-κB luciferase activities were measured after the cells were transfected with NF-kB luciferase promoter (&, #, $, P < 0.01 vs. previous column group).

4. Discussion

Due to the high cost of developing new agents for diabetic retinopathy, we adopted an alternative way to search for possible treatment benefits for this complication based on currently available anti-diabetic compounds, such as the gliptin family of agents. Surprisingly, our efforts paid off. Our data suggest that linagliptin is an optional agent which exerts potent vascular protection in retinal endothelial cells. Our data support that linagliptin is a pro-survival, anti-inflammatory, anti-ROS agent that protects RECs from injury induced by the cytokine TNF-α. The inhibitory effect of linagliptin on vascular cytokines and adhesion molecules can be explained by its ability to reduce adhesion of immune cells to RECs. Its suppression of ROS production is concordant with its ability to protect retinal endothelial mitochondrial function. Our data indicate that linagliptin potently inhibits cytokine-induced activation of NF-κB, which highlights the mechanism of the molecular pathway it regulates. It would be ideal to have an anti-diabetic agent that could exert broad retinal vascular protection in order to limit retinopathy, and DPP-4 inhibitors appears to meet this requirement.

Like other gliptin compounds, linagliptin acts by inhibiting the enzymatic activity of DPP-4, stabilizing incretins such as GLP-1, and ultimately lowering blood glucose. Linagliptin is unique among the gliptins. Its administration has a mild influence on hepatic function, and it is excreted largely by non-renal routes. Therefore, dose adjustment is not required in patients with renal and hepatic impairment [5]. Aside from its ability to lower blood glucose, linagliptin has been shown to be effective for cardiovascular outcomes [8]. In vascular endothelial cells, recent studies have shown that linagliptin has vascular benefits and these properties are independent of its glucose-lowering property. Multiple separate studies have shown that linagliptin treatment could prevent diabetes condition and generation of ROS and inflammatory genes including RAGE, ICAM-1, SOD2, and PAI-1 expression in endothelial cells induced by its byproducts [9–11]. Vellecco V et al. showed that linagliptin exerts a direct vasodilation activity on vessels from both normal and hyperglycemic mice, suggesting that the vascular effect of linagliptin is independent from its ability to control glucose. Additionally, cell experiments have concluded that linagliptin increases eNOS availability and enhances NO production [12]. Romacho T et al. found that linagliptin helps to preserve microvascular endothelial function by suppressing PAR2 activation and thromboxane A2 release [13].

Endothelial progenitor cells (EPCs) play key roles in the process of endothelial repair and replacement for vascular homeostasis and neoangiogenesis [14]. In type 2 diabetes patients, EPCs are known to be reduced. A recent study reported that linagliptin acutely increases the number of endothelial progenitor cells [15]. Endothelial-to-mesenchymal transition (EndMT) is the cellular process in which endothelial cells lose their identity as a result of environmental changes, and EndMT has been implicated in vascular remodeling and various disease conditions. Studies have shown that linagliptin possesses unique specific effects and can suppress transforming growth factor-β2-induced endothelial-mesenchymal transition [16,17]. Additionally, the versatility of linagliptin is reflected in its ability to inhibit angiogenesis signaling and reduce neovascularization in both HUVECs and brain vascular endothelial cells [18,19].

In preclinical animal model studies, linagliptin administration has been shown to possess vascular protective effects. Michurina et al. report that linagliptin administration could protect the hepatic microvasculature in a diabetic fatty liver mouse model [20]. Manrique et al. demonstrate that linagliptin administration could prevent high fat diet-induced aortic stiffening, vascular oxidative stress, endothelial dysfunction, and vascular remodeling [21]. Hardigan et al. report that linagliptin administration in diabetic rats reduces plama ET-1 levels and cerebrovascular hyperreactivity. This effect is potentially the result of linagliptin causing a decrease in endothelial TLR2 expression and a subsequent increase in NO bioavailability [22]. A study using an LPS-induced rat sepsis model showed that administration of linagliptin has pleiotropic vasodilatory, antioxidant, and anti-inflammatory properties [8]. In atherosclerosic mice, linagliptin ameliorates hyperlipidemia-induced endothelial dysfunction [18]. In humans, a randomized placebo-controlled study demonstrated that Linagliptin tends to improve endothelial and microvascular function in patients with type 2 diabetes [23].

Diabetic retinopathy is a chronic inflammatory disease. Local inflammation plays a pivotal role in the pathological development and progression of this disease [24]. In addition to TNF-α, several other

cytokines, chemokines, and other factors such as IL-1β, IL-6, and C-reactive protein have been indicated to be associated with the pathophysiology and the microvascular complications of diabetic retinopathy. These factors are elevated in the retina and vitreous of diabetic patients and animal models of diabetes [25]. In this study, we found that linagliptin treatment significantly reduced TNF-α-induced expression and secretion of IL-6 and IL-8. We also investigated the effects of linagliptin on IL-1β-induced insults in endothelial cells. Consistent results were found which displayed that linagliptin possesses a protective effect against IL-1β-induced THP-1 adhesion to retinal endothelial cells (RECs) (Results not shown). In the future, the synergistic contributions of redox-inflammatory processes for endothelial dysfunction in diabetic retinopathy are should be examined.

Taken together, the findings of this study indicate that the vascular protective effects of DPP-4 inhibitors can be broadly appreciated both in vitro and in vivo. Retinal ECs are highly differentiated and high energy demanding endothelial cells, which convert light to chemical signals to facilitate light sensing. The integrity and fine regulation is critical to preserving visual function in diabetic conditions. Our data support that DPP-4 inhibitors could possess unique vascular protection and its application could be a potential choice in the treatment of diabetic retinopathy.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2018.10.040.

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