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In Vitro Study Examining the Activity of Vildagliptin and Sitagliptin against Hyperglycemia-Induced Effects in Human Umbilical Vein Endothelial Cells

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Abstract

Background: In diabetes, endothelial dysfunction leads to vascular complications that negatively impact on patient outcome. Endothelial dysfunction may be mediated by hyperglycemia-induced oxidative stress, protein kinase C-β (PKC-β) activation and endoplasmic reticulum (ER) stress. As these events are antagonized by glucagon-like peptide-1 (GLP-1), a therapeutic strategy able to increase GLP-1 levels may prevent endothelial impairment, providing a clinical benefit to diabetic patients.

Aim of the present study was to test the capacity of dipeptidyl peptidase-4 (DPP-4) inhibitors, known to increase GLP-1 levels, to contrast hyperglycemia-induced effects in human umbilical vein endothelial cells (HUVECs).

Methods: HUVECs grown for 21 days under conditions of continuous normal or high glucose (NG and HG, respectively) were treated with the DDP-4 inhibitors vildagliptin and sitagliptin (5 nM), or with GLP-1 (50 nM, as control) for 1 h. After cell harvesting, the following markers were quantitated: reactive oxygen species, thioredoxin-interacting protein mRNA and PKC-β mRNA (oxidative stress); BAX and BCL-2 transcripts (apoptosis); BIP, CHOP and IRE-10 mRNA (ER stress) and GLP-1 receptor (GLP-1R).

Results: In our experimental model, compared to NG, HG confirmed to trigger a significant increase of oxidative stress, apoptosis and ER stress while reducing GLP-1R expression. Under NG conditions, no treatment exerted any effect. In contrast, when HUVECs grown under HG conditions were treated with DDP-4 inhibitors, a significant overall improvement was observed in terms of oxidative and ER stress and apoptosis reduction. Notably, vildagliptin's activity was slightly better than sitagliptin's and both exerted effects comparable to that of GLP-1.

Conclusions: DDP-4 inhibition by vildagliptin and sitagliptin had a protective action, similar to that of GLP-1, on HUVECs against HG-induced effects. Our preliminary findings suggest that, in diabetic patients, this strategy might be beneficial in contrasting endothelial dysfunction.

Keywords: Apoptosis; Endoplasmic reticulum stress; GLP-1; High glucose; Oxidative stress; Sitagliptin; Vildagliptin

Introduction

Glucagon-like peptide-1 (GLP-1) is a hormone released by the intestinal L cells in response to food intake. This mediator is rapidly degraded by dipeptidyl peptidase-4 (DPP-4), a ubiquitous transmembrane glycoprotein that cleaves N-terminal dipeptides from a variety of substrates. As a result, the half-life of circulating GLP-1 is 1-2 minutes. Besides its key role in the regulation of insulin secretion and glucose homeostasis [1], GLP-1 is also emerging as an important player in the protection of kidney and cardiovascular system [2-4]. Moreover, GLP-1 improves endothelial function, and this is particularly important in the context of diabetes, in which hyperglycemia and oxidative stress lead to endothelial dysfunction, ultimately resulting in vascular complications. Evidence suggests that in diabetes, due to continuous exposure to high levels of glucose, the protective action of GLP-1 is significantly decreased [5,6]. Two mechanisms have been proposed to explain this phenomenon: i) hyperglycemia-induced activation of protein kinase C beta (PKC-β), which is able to reduce the expression of GLP-1 receptors [6], and ii) hyperglycemia-generated oxidative stress [5]. Indeed, a recent *in vivo* study showed that the administration of the antioxidant vitamin C was able to improve both GLP-1 action and hyperglycemia control [5]. On the other hand, in endothelial cells, GLP-1 may counteract the high glucose-induced endoplasmic reticulum (ER) stress [7], which has been implicated in type 2 diabetes (T2D) and endothelial dysfunction [8,9]. Under ER stress, the unfolded protein response (UPR) is activated in order to restore normal ER homeostasis [8]. UPR acts by promoting the inhibition of protein synthesis, the degradation of misfolded proteins and the production of molecular chaperones involved in protein folding [8]. A key player of UPR is immunoglobulin protein (BIP), a chaperone that usually maintains in the inactive form the three transmembrane sensors of ER stress: protein kinase RNA-like ER kinase (PERK), inositol requiring enzyme 1α (IRE-1 α) and activating transcription factor–6. Under ER stress, BIP dissociates from the sensors, that can thus induce the expression of

different target genes, and retranslocates misfolded proteins [8]. In case of prolonged ER stress, PERK signaling triggers apoptosis [10]. In this context, one major event is the induction of CCAAT-enhancer-binding protein homologous protein (CHOP), that, in turn, modulates the expression of several BCL-2 family members [10,11].

In the past decade, therapeutic strategies aimed at increasing levels of circulating GLP-1 by inhibiting DPP-4 have been developed and shown to be effective in T2D [12,13]. Vildagliptin is a DPP-4 inhibitor that has displayed better antioxidant properties than other compounds in its class [12-14], significantly lowering oxidative stress-related markers. Still, to date, available data are poor.

The aim of this study was to investigate the anti-oxidant properties of vildagliptin, compared to another DDP-4 inhibitor, sitagliptin, on human umbilical vein endothelial cells (HUVECs) exposed to high levels of glucose. Moreover, the effects of both inhibitors on PKC-β activation and ER/UPR stress markers were evaluated.

Materials and Methods

Cell culture and experimental design

HUVECs were purchased from Lonza (Lonza Bioresearch LBS, Basel, Switzerland) and cultured with EGM™-2 Bulletkit™ (Lonza) supplemented with human epidermal growth factor (hEGF), hydrocortisone, human recombinant fibroblast growth factor-beta (hFGF-b), heparin, 2% fetal bovine serum (FBS), and gentamicin/amphotericin-B (GA), at 37°C in a humidified atmosphere with 5% CO_2.

Cells were used at passage 4 to avoid age-dependent cellular modifications, and plated in duplicate at a density of 2×10^5 in 6-well plates for total RNA and protein extraction, and at 1×10^4 in 96-well plates for reactive oxygen species (ROS) measurement. Briefly, after seeding, HUVECs were allowed to attach overnight and 1 day later were exposed to either normal (5 mM) or high (25 mM) glucose concentrations (NG and HG, respectively) and cultured for a further 21 days. Vildagliptin (5 nM), sitagliptin (5 nM) (Sigma-Aldrich, St Louis, MO, USA) or GLP-1 (50 nM) (Sigma-Aldrich) were added to cells 1 hour before cell harvesting for the subsequent analyses.

Measurement of ROS

The fluorescent probe 2′,7′-dichlorofluorescein diacetate (H2DCFDA, Invitrogen, Carlsbad, California, USA) was used to measure the intracellular generation of ROS, following the manufactur's instructions. After a 1 hr-incubation with the indicated drugs, cells were stained with 20 μM H2DCFDA for 30 min at 37°C. Fluorescence intensity of H2DCFDA was kinetically measured at an excitation and emission wavelength of 485 nm and 530 nm, respectively, using a fluorescent microplate reader (Synergy HT, BioTek Instruments, Inc., Winooski, Vermont, USA).

RNA isolation and qRT-PCR

Total RNA was isolated from HUVECs using a total RNA isolation kit (Norgen Biotek Corp, Thorold, Ontario, Canada) following the manufacturer's instructions. First-strand cDNA was prepared using 1-2 μg of total RNA, the Superscript III RT kit and random hexamer primers (Invitrogen, Carlsbad, CA, USA) in a total volume of 25 μl according to the manufacturer's instructions. Reverse transcription was carried out for 90 min at 50°C followed by 10 min at 55°C. qRT-PCR was performed on a QuantStudio 6 flex (Applied Biosystem) detection system using SybrGreen reagents (Takara Bio Company, Clontech, Mountain View, CA, USA) and TaqMan® Gene Expression Master Mix (Thermo Fisher Scientific, Waltham, MA, USA).

The expression of the following markers was evaluated (primers and conditions are listed in Table 1; thioredoxin-interacting protein (TXNIP) and PKC-β (oxidative stress), BAX and BCL-2 (apoptosis), and BIP, CHOP and IRE-1α (ER/UPR stress).

Gene	Sequence	Probe
TXNIP	GCAGTGCAAACAGACTTCGG	Sybr
	TCACCTGTTGGCTGGTCTTC	
β-ACTIN	CAGCCATGTACGTTGCTATCCAGG	Sybr
	AGGTCCAGACGCAGGATGGCATG	
BAX	TCAGGATGCGTCCACCAAGAAG	Sybr
	TGTGTCCACGGCGGCAATCATC	
BCL-2	ATCGCCCTGTGGATGACTGAGT	Sybr
	GCCAGGAGAAATCAAACAGAGGC	
CHOP	AGGGAGAACCAGGAAACGGAAACA	Sybr
	TCCTGCTTGAGCCGTTCATTCTCT	
IRE-1 α	CCGGCCTCGGGATTTTTGG	Sybr
	TTTGATTGAGCCTGTCCTC	
BIP	GACCTGGGGACCACCTACTC	Sybr
	TTCAGGAGTGAAGGCGACAT	
PKC-β	Hs00176998_m1	TaqMan
β-ACTIN	Hs99999903_m1	TaqMan

Table 1: Primers used to assess the transcript levels of the indicated genes by quantitative-Real Time-PCR, in HUVECs either untreated or treated with vildagliptin or sitagliptin upon different glucose conditions.

Protein analysis

For western blot analysis, HUVECs were lysed in RIPA buffer (Sigma-Aldrich) with 10% protease and 1% phosphatase inhibitors (Sigma-Aldrich). Protein content was determined using the Bradford assay buffer (Sigma-Aldrich), and 50 μg of lysates were separated by electrophoresis using 4-12% PAGE gels (Lonza) and transferred onto a PVDF membrane (Perkin Elmer, Waltham, MA, USA). After blocking with 5% non-fat dried milk or 5% bovine serum albumin, membranes were incubated with primary antibodies 1:500 anti-GLP-1 receptor (GLP-1R, Abcam, Cambridge, UK) and 1:1,000 anti-β-actin (Sigma-Aldrich, St Louis, MO, USA) overnight at 4°C. After incubation with the appropriate secondary horseradish peroxidase-conjugated IgG antibodies (GE Healthcare Europe GmbH, Milan, Italy) at a 1:3,000 dilution for 1 h at room temperature, blots were visualized by LI-COR ECL Reagent and C-DiGit Blot scanner (LI-COR Biosciences). Proteins were quantitated by the Image Studio software (www.licor.com). β-actin served as the loading control.

Statistical analysis

All experiments were repeated three times. Numerical data were expressed as mean ± *standard error* of the mean (SEM). Statistical analysis was performed using the one-way analysis of variance (ANOVA) with appropriate post-hoc multi-comparison tests (Tukey's) to compare the effects of vildagliptin, sitagliptin and GLP-1. A P-value of <0.05 was considered statistically significant.

Results

Effect of DDP-4 inhibitors on hyperglycemia-induced ROS generation

ROS generation was assessed by a fluorometric assay in HUVECs cultured for 21 days under NG or HG conditions and then exposed for 1h to vildagliptin, sitagliptin or GLP-1. As shown in Figure 1, compared to NG, HG significantly increased the production of ROS in untreated cells of approximately 3 fold (p<0.001). However, following treatment, the content of ROS significantly decreased under HG but not under NG, and the reduction was slightly lower following vildagliptin (P<0.05) compared to sitagliptin and GLP-1 (P<0.01).

Effect of DDP-4 inhibitors on hyperglycemia-induced TXNIP up-regulation

Compared to NG, HG significantly up-regulated by (approximately 3-fold) TXNIP transcript in untreated HUVECs (P<0.001) (Figure 2). Yet, this increase was partly reversed by 1h-exposure to vildagliptin, sitagliptin and GLP-1, but the significance was reached only upon vildagliptin and GLP-1 (P<0.05). Moreover, the levels of TXNIP mRNA observed in vildagliptin- and sitagliptin-treated cells remained significantly higher than those in the corresponding conditions upon NG (P<0.05). No change was observed following any treatment under NG conditions.

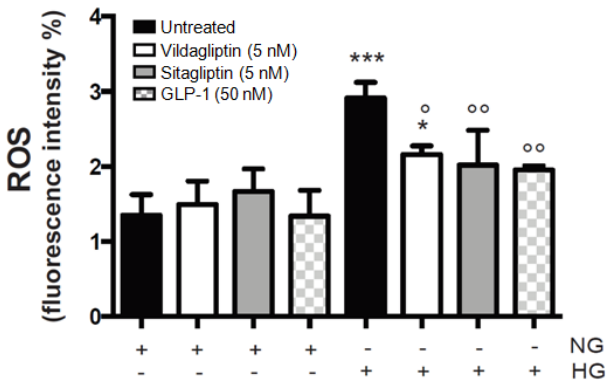

Figure 1: Generation of reactive oxygen species (ROS). Total ROS intensity measurement was performed in HUVECs after 21 days in culture under conditions of NG or HG. Cells were treated with vildagliptin (5 nM) or sitagliptin (5 nM) or GLP-1 (50 nM). Bars represent the mean ± SEM, n=3. One-way ANOVA, followed by Tukey's post-hoc test, where * *vs.* NG, ° *vs.* HG. *p<0.05, **p<0.01, ***p<0.001.

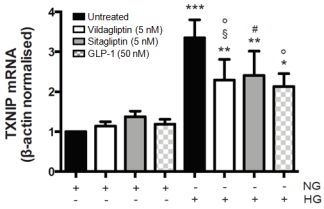

Figure 2: Gene expression of oxidative stress marker. Total cellular RNA was isolated from HUVECs after 21 days in culture under conditions of NG or HG. Cells were treated with vildagliptin (5 nM) or sitagliptin (5 nM) or GLP-1 (50 nM). The level of mRNA encoding for TXNIP was assessed by qRT-PCR and expressed relative to the level of β-actin. The experiment was repeated in triplicate. Bars represent the mean ± SEM. One-way ANOVA, followed by Tukey's post-hoc test, where * *vs.* NG, § vs Vildagliptin, ° *vs.* Sitagliptin, and ° *vs.* HG. *p<0.05, **p<0.01, ***p<0.001, §p<0.05, °p<0.05, # p<0.05.

Effect of DDP-4 inhibitors on hyperglycemia-induced deregulation of apoptosis-related markers

We next investigated the levels of the apoptotic markers BAX (pro-apoptotic) and BCL-2 (anti-apoptotic) by qRT-PCR (Figure 3). In untreated cells, compared to NG, HG led to significant BAX up-regulation (p<0.01) (Figure 3A), but no BCL-2 change (Figure 3B). Under HG conditions, treatment with vildagliptin, sitagliptin and GLP-1 significantly decreased BAX transcript levels (p<0.05), restoring similar levels observed upon NG (Figure 3A). Conversely, exposure to any drug upon HG led to significant BCL-2 up-regulation (p<0.001 upon vildagliptin; p<0.01 upon sitagliptin and GLP-1) (Figure 3B).

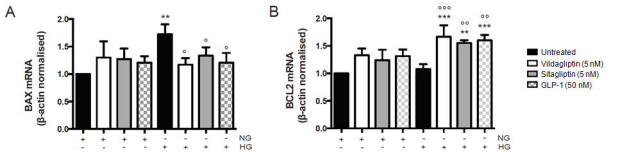

Figure 3: Up-regulation of apoptosis-related markers revealed Vildagliptin anti-apoptotic effect. Total cellular RNA was isolated from HUVECs after 21 days in culture in NG or HG. Cells were treated with vildagliptin (5 nM) or sitagliptin (5 nM) or GLP-1 (50 nM). The level of mRNA encoding for BAX (A) and BCL2 (B) was assessed by qRT-PCR and expressed relatively to β-actin. Bars represent the mean±SEM, n=3. One-way ANOVA, followed by Tukey's post-hoc test, where * *vs.* NG and ° *vs.* HG. **p<0.01, ***p<0.001, °p<0.05, °°p<0.01, °°°p<0.001.

Effect of DDP-4 inhibitors on hyperglycemia-induced PKC-β transcript up-regulation

PKC-β is known to induce cellular ROS and oxidative stress in diabetes. Prolonged exposure of HUVECs to HG induced an increase of PKC-β transcript levels of nearly 2 fold (p<0.001) (Figure 4). Upon administration of vildagliptin, sitagliptin and GLP-1, however, a significant reduction was observed compared to untreated cells

(p<0.01, p<0.01 and p<0.001, respectively), and all agents restored levels comparable to those found upon NG. In contrast, under NG conditions, none of the drugs exerted any effect on PKC-β expression.

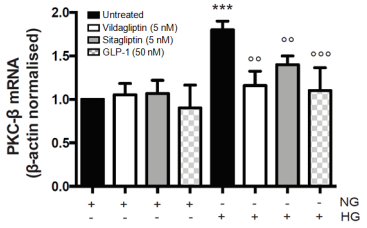

Figure 4: PKC-β activation. Total cellular RNA was isolated from HUVECs after 21 days in culture in NG, HG. Cells were treated with Vildagliptin (5 nM) or Sitagliptin (5 nM) or GLP-1 (50 nM). mRNA encoding for PKC-β was assessed by qRT-PCR and expressed relatively to β-actin. Bars represent mean ± SEM. One-way ANOVA, followed by Tukey's post-hoc test, where * *vs.* NG and ° *vs.* HG. ***p<0.001 and °°p<0.01.

Effect of DDP-4 inhibitors on hyperglycemia-induced ER/UPR gene expression

The transcript level of the well-established markers of ER stress BIP, CHOP and IRE-1α was assessed. Prolonged exposure to HG, compared to NG, significantly increased their levels in untreated cells, and did so to a similar extent (p<0.001 in each case) (Figure 5).

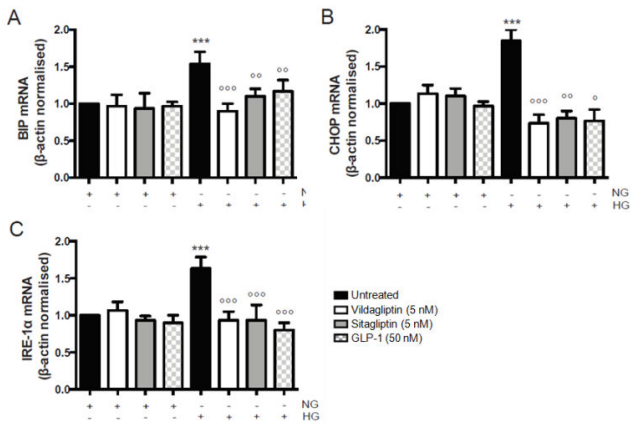

Figure 5: Gene expression of ER/UPR stress markers. (A-C) Total cellular RNA was isolated from HUVECs after 21 days in culture in NG, HG. Cells were treated with Vildagliptin (5 nM) or Sitagliptin (5 nM) or GLP-1 (50 nM). mRNA encoding for BIP, CHOP and IRE-1α was assessed by qRT-PCR and expressed relatively to β-actin. Bars represent mean ± SEM. One-way ANOVA, followed by Tukey's post-hoc test, where * *vs.* NG and ° *vs.* HG. ***p<0.001, °p<0.05, °°p<0.01 and °°°p<0.001.

However, treatment with vildagliptin, sitagliptin and GLP-1 led to a significant reduction of all markers down to levels comparable to those observed upon NG (p<0.001 in all cases except for sitagliptin and GLP-1 on BIP levels: p<0.01) (Figure 5). Under NG conditions, no treatment exerted any effect on BIP, CHOP and IRE-1α expression.

Effect of DDP-4 inhibitors on hyperglycemia-induced down-regulation of GLP-1 receptor

The expression of GLP-1R was assessed by Western blotting. A significant down-regulation was observed in untreated cells exposed to HG compared to those grown with NG (by approximately 50%, p<0.05) (Figure 6). Compared to untreated cells, each drug was able to significantly increase the expression of GLP-1R in HG (p<0.01), restoring levels similar to those observed under NG. Under NG conditions, no significant difference occurred upon any drug compared to untreated cells.

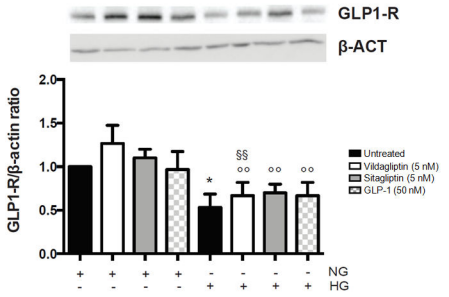

Figure 6: Protein expression of GLP-1R in HUVECs. Total whole cell lysate was isolated from HUVECs. Equal levels of protein lysates for GLP-1R were resolved on SDS-PAGE and expressed to relative β-actin expression, n=3. Bars represent mean ± SEM. One-way ANOVA, followed by Tukey's post-hoc test, where * *vs.* NG, § vs Vildagliptin and ° *vs.* HG. *p<0.05, °°p<0.01 and §§p<0.01.

Discussion

DPP-4 inhibitors are effective drugs for the treatment of T2DM [12,13]. They act by increasing the levels of biologically intact GLP-1, which, in turn, enhances glucose metabolism through the up-regulation of insulin secretion and the suppression of glucagon release. Moreover, GLP-1 protects endothelial cells from hyperglycemia-induced dysfunction that may be caused by oxidative stress. Vildagliptin is a DPP-4 inhibitor that displays more prolonged DPP-4 enzyme inhibition (acting as a substrate-blocker) compared to sitagliptin (acting, conversely, as a competitive inhibitor), thereby resulting in higher GLP-1 plasma levels upon the former compared to the latter [14-16].

In the present study, we tested the effects of both DDP-4 inhibitors on different hyperglycemia-induced events in HUVECs. Results showed that vildagliptin, as well as sitagliptin, can reverse, at least in part, the effects triggered by the prolonged exposure to HG. Indeed, they were able to restore, for most of the markers examined, levels down to those observed under NG. We previously tested the effects of another DDP-4 inhibitor, teneligliptin, on these markers, and compared its activity to that of sitagliptin [7]. Results were similar to the findings reported here, further confirming the protective effect exerted by DDP-4 inhibition on endothelial cells, at least for the markers taken into account. Although we did not assess the activity of DDP-4 following treatment with vildagliptin and sitagliptin, we used

50 nM GLP-1 as a positive control throughout the study, and observed comparable effects by the three agents. In particular, they affected the levels of the GLP-1R protein, a mediator of most of the main effects of GLP-1, to a similar extent upon both NG and HG, suggesting that these two inhibitors actually act by increasing the levels of endogenous GLP-1.

It is well established that, upon continuous exposure to HG, PKC-β activation occurs, that triggers oxidative stress [17]. This, in turn, leads to increased apoptosis and reduced protection of endothelial cells by GLP-1, leading to dysfunction and finally to diabetic vascular complications [7,18,19]. In our experimental model, compared to NG conditions, exposure to HG actually increased the content of ROS and the expression of the oxidative stress marker TXNIP, of PKC-β, of ER/UPR stress markers BIP, CHOP and IRE-1α and of the pro-apoptotic BAX. Conversely, it decreased the expression of GLP-1R, while exerting no effect on the expression of BCL-2. 1h-treatment with GLP-1 was able to significantly counteract these events, and the exposure to vildagliptin and sitagliptin, which increase the levels of endogenous GLP-1 by inhibiting DDP-4, led to similar results, pointing toward their antioxidant capacity, confirming our previous findings [7]. Moreover, our findings show that vildagliptin and sitagliptin may counteract the overall pro-apoptotic effect induced by HG, stimulating BCL-2 and reducing BAX expression at the same time. Notably, it has been reported that, upon induction of prolonged ER stress-mediated apoptosis, CHOP is up-regulated and modulates the levels of different mediators of apoptosis, such as Bcl-2 [11] and Bax [20].

It has been proposed that GLP-1 could restore HG-induced ER stress in endothelial cells. Impairment of ER function triggers ER stress, leading to UPR activation (through BIP, CHOP and IRE-1α), which causes inhibition of protein synthesis, protein refolding, and clearance of misfolded proteins. Although each UPR pathway activates different target genes, the overall result is that the cell can counteract the endogenous stress of unfolded proteins by shutting off the synthesis of new proteins [16]. It is well known that HG induces the expression of ER stress-related genes, and this was confirmed also in our model. Notably, vildagliptin and sitagliptin had the capacity to down-regulate the expression level of BIP, CHOP and IRE-1α in HUVECs cultured under HG, thus reversing HG-induced ER stress. Recently, it has been shown that the expression of GLP-1R was reduced in HUVECs exposed to HG, but could be partially restored by a PKC-β-specific inhibitor [7]. Interestingly, upon HG, we observed an increase of PKC-β transcript and a decrease of GLP-1R protein level.

This study does present with some limitations. First, following treatment with vildagliptin and sitagliptin, we evaluated apoptosis induction by BAX and BCL2 transcript levels, but not HUVEC viability and proliferation. We focused on apoptosis as it is a well-established effect triggered by hyperglycemia [21]. Accordingly, it has been previously shown that HG-induced apoptosis in HUVECs is accompanied by increased Bax/Bcl_2 ratio [21-23], that can be reverted, at least partly, by treatment with DDP-4 inhibitors [7]. Second, we did not investigate the possible effects of DDP-4 inhibitors on nitric oxide synthase (eNOS) expression and activity, as it was well beyond the scope of this study and *in vitro* and *in vivo* studies have already provided evidence linking GLP-1, DDP-4 inhibitors, eNOS and endothelial function [24-28]. Impairment of eNOS is recognized as the major mechanism leading to macrovascular complications in T2DM. Notably, this enzyme has been implicated in GLP-1-induced vasorelaxation *in vivo* [24] and, in HUVECs, GLP-1 increased eNOS protein expression and activity through GLP-1R-dendent and -independent mechanisms [25]. In ischemic mice, vildagliptin has been recently shown to promote revascularization through eNOS activation [28] and sitagliptin enhanced the levels of circulating EPC and neovasculogenesis in an eNOS-dependent fashion [27]. These results warrant further investigations, also to better clarify the possible different cardiovascular effects of the different agents currently employed in the treatment of diabetic patients. Moreover, it is necessary to yield more definitive information on the protective and antioxidant capacity of DDP-4 inhibitors by focusing on the expression profile of different markers, as well as on the improvement of the assessment of the cellular redox state.

In conclusion, our findings provide evidence supporting the protective action of vildagliptin and sitagliptin against HG-induced processes on endothelial cells. Notably, their effects mimic those of GLP-1, which is known to play a key role in the maintenance of CV system homeostasis.

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Authorship Contributions

LLS contributed to the acquisition, analysis and interpretation of data and manuscript writing. SG and AC contributed to analysis and interpretation of data and manuscript writing. All authors have read and approved the final manuscript.

References

1. Ussher JR, Drucker DJ (2012) Cardiovascular biology of the incretin system. Endocr Rev 33: 187-215.
2. Forst T, Weber MM, Pfützner A (2012) Cardiovascular Benefits of GLP-1-BasedTherapies in Patients with Diabetes Mellitus Type 2: Effects on Endothelial and Vascular Dysfunction beyond Glycemic Control.
3. Park CW, Kim HW, Ko SH, Lim JH, Ryu GR, et al. (2007) Long-term treatment of glucagon-like peptide-1 analog exendin-4 ameliorates diabetic nephropathy through improving metabolic anomalies in db/db mice. J Am Soc Nephrol 4: 1227-1238.
4. Kodera R, Shikata K, Kataoka HU, Takatsuka T, Miyamoto S, et al. (2011) Glucagon-like peptide-1 receptor agonist ameliorates renal injury through its anti-inflammatory action without lowering blood glucose level in a rat model of type 1 diabetes. Diabetologia 4: 965-978.
5. Ceriello A, Esposito K, Testa R, Bonfigli AR, Marra M, et al. (2011) The possible protective role of glucagon-like peptide 1 on endothelium during the meal and evidence for an "endothelial resistance" to glucagon-like peptide 1 in diabetes. Diabetes Care 34: 697-702.
6. Mima A, Hiraoka-Yamomoto J, Li Q, Kitada M, Li C, et al. (2012) Protective effects of GLP-1 on glomerular endothelium and its inhibition by PKC-beta activation in diabetes. Diabetes 61: 2967-2979.
7. Pujadas G, De Nigris V, Prattichizzo F, Sala LL, Testa R, et al. (2016) The dipeptidyl peptidase-4 (DPP-4) inhibitor teneligliptin functions as antioxidant on human endothelial cells exposed to chronic hyperglycemia and metabolic high-glucose memory. Endocrine .
8. Lenna S, Han R, Trojanowska M (2014) Endoplasmic reticulum stress and endothelial dysfunction. IUBMB Life 66: 530-537.
9. Amin A, Choi SK, Galan M, Kassan M, Partyka M, et al. (2012) Chronic inhibition of endoplasmic reticulum stress and inflammation prevents ischaemia-induced vascular pathology in type II diabetic mice. J Pathol 2: 165-174.

10. Urra H, Dufey E, Lisbona F, Rojas-Rivera D, Hetz C (2013) When ER stress reaches a dead end. Biochim Biophys Acta 1833: 3507-3517.
11. McCullough KD, Martindale JL, Klotz LO, Aw TY, Holbrook NJ (2001) Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state. Mol Cell Biol 21: 1249-1259.
12. Forst T, Bramlage P (2014) Vildagliptin , a DPP-4 inhibitor for the twice-daily treatment of type 2 diabetes mellitus with or without metformin. Expert Opin Pharmacother 15: 1299-1313.
13. Duvnjak L, Blaslov K (2016) Dipeptidyl peptidase-4 inhibitors improve arterial stiffness, blood pressure, lipid profile and inflammation parameters in patients with type 2 diabetes mellitus. Diabetol Metab Syndr 8: 26.
14. Rizzo MR, Barbieri M, Marfella R, Paolisso G (2012) Reduction of oxidative stress and inflammation by blunting daily acute glucose fluctuations in patients with type 2 diabetes: role of dipeptidyl peptidase-IV inhibition. Diabetes Care 10: 2076-2082.
15. Ahrén B, Schweizer A, Dejager S, Villhauer EB, Dunning BE, et al. (2011) Mechanisms of action of the dipeptidyl peptidase-4 inhibitor vildagliptin in humans. Diabetes Obes Metab 13: 775-783.
16. Davis JA, Singh S, Sethi S, Roy S, Mittra S, et al. (2010) Nature of action of Sitagliptin, the dipeptidyl peptidase-IV inhibitor in diabetic animals. Indian J Pharmacol 42: 229-233.
17. Koya D, King GL (1998) Protein kinase C activation and the development of diabetic complications. Diabetes 47: 859-866.
18. Sasaki S, Inoguchi T (2012) The role of oxidative stress in the pathogenesis of diabetic vascular complications. Diabetes Metab J 36: 255-261.
19. Quagliaro L, Piconi L, Assaloni R, Martinelli L, Motz E, et al. (2003) Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD(P)H-oxidase activation. Diabetes 11: 2795-2804.
20. Fu HY, Okada K, Liao Y, Tsukamoto O, Isomura T, et al. (2010) Ablation of C/EBP homologous protein attenuates endoplasmic reticulum-mediated apoptosis and cardiac dysfunction induced by pressure overload. Circulation 4: 361-369.
21. van den Oever IA, Raterman HG, Nurmohamed MT, Simsek S (2010) Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. Mediators Inflamm 2010: 792393.
22. Risso A, Mercuri F, Quagliaro L, Damante G, Ceriello A (2001) Intermittent high glucose enhances apoptosis in human umbilical vein endothelial cells in culture. Am J Physiol Endocrinol Metab 281: E924-930.
23. Yang Z, Mo X, Gong Q, Pan Q, Yang X, et al. (2008) Critical effect of VEGF in the process of endothelial cell apoptosis induced by high glucose. Apoptosis 13: 1331-1343.
24. Ban K, Noyan-Ashraf MH, Hoefer J, Bolz SS, Drucker DJ, et al. (2008) Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation 117: 2340-2350.
25. Ding L, Zhang J (2012) Glucagon-like peptide-1 activates endothelial nitric oxide synthase in human umbilical vein endothelial cells. Acta Pharmacol Sin 33: 75-81.
26. Fadini GP, Boscaro E, Albiero M, Menegazzo L, Frison V, et al. (2010) The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal-derived factor-1alpha. Diabetes Care 7: 1607-1609.
27. Huang CY, Shih CM, Tsao NW, Lin YW, Huang PH, et al. (2012) Dipeptidyl peptidase-4 inhibitor improves neovascularization by increasing circulating endothelial progenitor cells. Br J Pharmacol 7: 1506-1519.
28. Ishii M, Shibata R, Kondo K, Kambara T, Shimizu Y, et al. (2014) Vildagliptin stimulates endothelial cell network formation and ischemia-induced revascularization via an endothelial nitric-oxide synthase-dependent mechanism. J Biol Chem 39: 27235-27245.