EXPERIMENTAL STUDY

Vildagliptin, a DPP-4 Inhibitor, Attenuates Endothelial Dysfunction and Atherogenesis in Nondiabetic Apolipoprotein E-Deficient Mice

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Summary

Dipeptidyl peptidase-4 (DPP-4) inhibitors are novel antidiabetic agents with possible vascular protection effects. Endothelial dysfunction is an initiation step in atherogenesis. The purpose of this study was to investigate whether vildagliptin (Vilda) attenuates the development of endothelial dysfunction and atherosclerotic lesions in nondiabetic apolipoprotein E-deficient (ApoE−/−) mice. Eight-week-old nondiabetic ApoE−/− mice fed a Western-type diet received Vilda (50 mg/kg/day) for 20 weeks or 8 weeks. After 20 weeks of treatment, Vilda administration reduced atherogenesis in the aortic arch as determined by en face Sudan IV staining compared with the vehicle group (P < 0.05). Vilda also reduced lipid accumulation (P < 0.05) and vascular cell adhesion molecule-1 (VCAM-1) expression (P < 0.05) and tended to decrease macrophage infiltration (P = 0.05) into atherosclerotic plaques compared with vehicle. After 8 weeks of treatment, endothelium-dependent vascular reactivity was examined. Vilda administration significantly attenuated the impairment of endothelial function in nondiabetic ApoE−/− mice compared with the vehicle group (P < 0.05). Vilda treatment did not alter metabolic parameters, including blood glucose level, in both study protocols. To investigate the mechanism, aortic segments obtained from wild-type mice were incubated with exendin-4 (Ex-4), a glucagon-like peptide-1 (GLP-1) analog, in the presence or absence of lipopolysaccharide (LPS). Ex-4 attenuated the impairment of endothelium-dependent vasodilation induced by LPS (P < 0.01). Furthermore, Ex-4 promoted phosphorylation of eNOS at Ser1177 which was decreased by LPS in human umbilical endothelial cells (P < 0.05). Vilda inhibited the development of endothelial dysfunction and prevented atherogenesis in nondiabetic ApoE−/− mice. Our results suggested that GLP-1-dependent amelioration of endothelial dysfunction is associated with the atheroprotective effects of Vilda.

Key words: Diabetes mellitus, Endothelial function, Atherosclerosis, GLP-1

Recent animal and clinical studies have documented that the cardiovascular protective properties of dipeptidyl peptidase-4 (DPP-4) inhibitors are independent of their antidiabetic action.1,4 The fundamental role of DPP-4 inhibitors is elevation of glucagon-like peptide-1 (GLP-1) level, which promotes insulin secretion from the pancreas,1,7 whereas the receptor for GLP-1 is widely expressed in many cell types, including vascular cells and macrophages, suggesting pleiotropic and cardioprotective effects of DPP-4 inhibitors beyond their blood glucose-lowering effect.6,7 Previous studies, including our own, have demonstrated that administration of DPP-4 inhibitors reduced the development of atherosclerotic plaques in normoglycemic animal models, with no alteration of metabolic parameters, including blood glucose and lipid levels.8-12 Several studies have also reported cardioprotective effects of DPP-4 inhibitors in clinical situations.13,14 The underlying mechanisms are not fully understood; however, several studies have demonstrated that inhibition of pro-inflammatory activation of vascular cells by GLP-1 contributes to the cardioprotective effects of DPP-4 inhibitors.6,13

Vildagliptin (Vilda), which is one of the most investigated DPP-4 inhibitors, shows a more stable glycemic...
control profile in diabetic patients compared with other members of this class. Previous studies have demonstrated that Vilda attenuated the progression of atherosclerosis in both diabetic and nondiabetic apolipoprotein E-deficient (ApoE−/−) mice by reduction of pro-inflammatory activation of macrophages, an important cell type in atherogenesis. Atherosclerosis is an inflammatory disease in which multiple cell types are involved. Vascular inflammation causes endothelial dysfunction, an initiator of atherosclerosis. Endothelial dysfunction alters vascular responses, which stimulate the development of atherosclerosis. Recent studies have suggested that endothelial dysfunction could be a therapeutic target for the inhibition of atherosclerotic disease. However, the effects of Vilda on endothelial cell function have not been fully investigated. Therefore, in this study, we administered Vilda to nondiabetic ApoE−/− mice and examined its effects on endothelial cell function and atherogenesis. Our findings demonstrated that Vilda reduces the development of atherosclerosis and ameliorates endothelial dysfunction in this mouse model. The results of in vitro and ex vivo experiments suggested that protective properties on endothelial cells which depend on GLP-1 at least partially contribute to these effects.

Methods

Animals and drug administration: ApoE−/− (C57BL/6J background) mice were originally purchased from the Jackson Laboratory. Mice were maintained under a 12-hour light/dark cycle. Vilda was supplied by Novartis Pharma. To examine the effect of Vilda on the development of atherosclerosis, male ApoE−/− mice were treated with Vilda 50 mg/kg/day from 8 weeks old by gavage for 20 weeks. To investigate the effect of Vilda on endothelial function at an earlier stage of atherosclerosis, the same dose of Vilda was administered to female ApoE−/− mice for 8 weeks. A Western-type diet (WTD) was started from 8 weeks old in both experiments. Vilda was dissolved in 0.5% carboxymethyl cellulose (CMC) solution. The control group received an equal volume of CMC. All experimental procedures conformed to the guidelines for animal experimentation of Tokushima University. The protocol was reviewed and approved by our institutional ethics committee.

Blood pressure and laboratory data: Blood pressure (BP) of each mouse was measured using a tail-cuff system (BP-98A, Softron) as described in our previous paper. The blood glucose level was measured from the tail vein using a glucometer (NIPRO StatStrip XP2, NIPRO) without fasting and with fasting in 8-week and 20-week treatments, respectively. At the time of sacrifice, blood was collected from the heart into EDTA-containing tubes. After blood samples were centrifuged, plasma was stored at −80°C until required. Plasma total cholesterol, high density lipoprotein (HDL)-cholesterol, and triglyceride levels were measured at LS1 Medience Corporation (Japan).

Quantification of atherosclerotic lesions: The development of atherosclerotic lesions in the aorta was determined by Sudan IV staining as described previously. In brief, mice were sacrificed with an overdose of pentobarbital and perfused with 0.9% sodium chloride solution at a constant pressure. Both the heart and whole aorta were immediately removed. The thoracic aorta was opened longitudinally and fixed with 10% neutral buffered formalin. To quantify atherosclerotic lesions in the aortic arch, we performed en face Sudan IV staining. The percentage of Sudan IV-positive area in the aortic arch was calculated. Histological and immunohistochemical analysis: The heart was cut along a horizontal plane between the lower tips of the left and right atria. The upper portion was snap-frozen in OCT compound (Tissue-Teck). Then, the aortic root was sectioned serially (at 5-μm intervals) from the point where the aortic valves appeared to the ascending aorta until the valve cusps were no longer visible. These frozen sections of the aortic root were used for histological and immunohistochemical analyses. Sections were stained with oil red O to detect lipid deposition. Also, sections were incubated with anti-CD31 antibody (MOMA-2) antibody (BioRad), anti-intercellular adhesion molecule-1 (ICAM-1) antibody, and anti-vascular cell adhesion molecule-1 (VCAM-1) antibody (Abcam). Sections were then incubated with biotinylated secondary antibody (VECTOR Laboratories, Inc.), followed by VECTASTAIN ABC-AP Kit (VECTOR Laboratories, Inc.), and stained using a VectorRed AP Substrate Kit (VECTOR Laboratories, Inc.). All sections were counterstained with hematoxylin. The ratio of positive area to plaque area was calculated in three valve lesions in the aortic root and used for comparison.

Vascular reactivity assay: The descend thoracic aorta was cut into 2-mm rings with special care to preserve the endothelium and mounted in an organ bath filled with modified Krebs-Henseleit buffer (KHB; 118.4 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM NaHCO3, 11.1 mM glucose) aerated with 95% O2 and 5% CO2 at 37°C. The preparations were incubated for 30 minutes.

Cell culture: Human umbilical vein endothelial cells (HUVEC) were purchased from Life Technologies and cultured in EGM-2 (Lonza). HUVEC (passages 4-6) were treated with 10 nM Ex-4 for 16 hours in EBM-2 containing 2% FBS and then stimulated with 10 ng/mL LPS for 30 minutes.

Western blot analysis: Cell lysates were prepared using RIPA buffer (Wako Pure Chemical Industries, Ltd.) containing a protease inhibitor cocktail (Takara Bio Inc.) and phosphatase inhibitors (Roche Life Science). Proteins were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Hybond-P; GE Health-
Vilda inhibited the development of atherosclerosis in nondiabetic ApoE−/− mice: To examine the effect of Vilda on the progression of atherogenesis, ApoE−/− mice were treated with Vilda or vehicle for 20 weeks. Vilda attenuated atherosclerotic lesion progression in the aortic arch as determined by en face Sudan IV staining compared with vehicle (P < 0.05) (Figure 1). Administration of Vilda to nondiabetic ApoE−/− mice did not alter metabolic parameters, including blood glucose and lipid levels, as shown in Table I. The result of oil red O staining demonstrated that Vilda significantly reduced lipid deposition in atherosclerotic plaques (P < 0.05) (Figure 2A). The result of immunostaining demonstrated that Vilda significantly reduced VCAM-1 expression (P < 0.05) (Figure 2A) and tended to decrease macrophage accumulation (P = 0.05) in atherosclerotic plaques (Figure 2B and C).

Vilda improved endothelial function in nondiabetic ApoE−/− mice: To investigate the effect of Vilda on endothelial function in a nondiabetic condition, vascular relaxation in response to Ach was significantly impaired in ApoE−/− mice compared with vehicle-treated mice. However, treatment with Vilda for 8 weeks significantly improved the impairment of endothelium-dependent vasodilation in ApoE−/− mice compared with vehicle administration (P < 0.05) (Figure 3A). On the other hand, endothelium-independent vasorelaxation in response to SNP did not differ between the Vilda and vehicle groups (Figure 3B). Metabolic parameters, including blood glucose level, did not differ between the Vilda-treated group and vehicle-treated group (Table II).

Ex-4 attenuated endothelial dysfunction induced by LPS: To investigate whether increased GLP-1 level is associated with improvement of endothelium-dependent vascular function, Ach-induced vasorelaxation was examined using aortic rings obtained from wild-type mice. Inflammatory stimulation with LPS impaired vasorelaxation in response to Ach, although Ex-4, a GLP-1 analog, significantly ameliorated this response (P < 0.01) (Figure 4A). Neither LPS nor Ex-4 affected endothelium-independent vasorelaxation in response to SNP (Figure 4B). To investigate the underlying mechanism by which Ex-4 attenuated impairment of endothelium-dependent vasorelaxation induced by LPS, we examined the phosphorylation of eNOS (Ser1177) in HUVEC. The results of western blotting demonstrated that phosphorylation of eNOS (Ser1177) was promoted by the presence of Ex-4 in LPS-treated HUVEC (P < 0.05) (Figure 5).

Discussion

In this study, we found that Vilda attenuated endothelial dysfunction and reduced atherosclerotic lesions in nondiabetic ApoE−/− mice. Vilda also reduced VCAM-1 expression and tended to decrease macrophage accumulation in atherosclerotic plaques. The results of an ex vivo experiment using aortic rings demonstrated that Ex-4, a GLP-1 analog, ameliorated endothelial dysfunction induced by LPS. Also, an in vitro experiment using HUVEC showed that Ex-4 increased eNOS (Ser1177) phosphorylation, which was deteriorated by LPS. Recent studies demonstrated protective effects of DPP-4 inhibitors on endothelial function. However, only a few studies have examined the effects of DPP-4 inhibitors on endothelial function and atherogenesis in a normoglycemic atherosclerotic mouse model.20-22 Also, we demonstrated that GLP-1 attenuated endothelial dysfunction in HUVEC stimulated with LPS, which plays an important role in the process of atherogenesis.23-25 The results of our study suggest that the elevated GLP-1 level caused by DPP-4 inhibition by Vilda contributes, at least partially, to the improvement of endothelial function and reduction of atherosclerotic lesion development.

Previous studies have demonstrated that DPP-4 inhibitors attenuate atherosclerosis in diabetic ApoE−/− mice.21,26 Atherosclerosis is the most serious manifestation in patients with diabetes. Blood glucose-lowering treatment with DPP-4 inhibitors suppresses multiple cellular and molecular mechanisms that stimulate atherogenesis. In fact, several clinical studies reported that DPP-4 inhibitors, including Vilda, improved endothelial dysfunction, an initiation step in the development of atherosclerosis in diabetic patients.24,27 On the other hand, accumulating evidence suggests that DPP-4 inhibitors prevent atherosclerosis independent of their blood glucose-lowering effect.8,17 One of the underlying mechanisms is the suppression of inflammatory activation of immune cells, such as macrophages.10,28-30 In addition to the activation of inflammatory cells, endothelial dysfunction plays a pivotal role in the initiation of atherogenesis.31 Therefore, in this study, we examined the effect of Vilda on the development of endothelial dysfunction and atherosclerosis in nondiabetic ApoE−/− mice. We found that Vilda-treated animals had reduced atherosclerotic lesions in the aortic arch and less accumulation of lipid and macrophages and inflammatory molecule expressions such as VCAM-1 in atherosclerotic plaques compared with vehicle-treated mice. These results are consistent with previous studies which reported atheroprotective effects of this class of antidiabetic drug.

Impaired endothelial function causes atherosclerosis.20 Endothelium-derived nitric oxide (NO) plays a cru-
Figure 1. Vilda inhibited the development of atherosclerosis in nondiabetic ApoE−/− mice. *En face* Sudan IV staining of the aortic arch showed that Vilda administration for 20 weeks significantly reduced the progression of atherosclerotic lesions in the aortic arch compared with the vehicle group (n = 10-12, per group). Scale bar: 1 mm.

Table 1. Effect of Vilda on Metabolic Parameters after 20-Week Treatment

| Parameter                      | Vehicle (n = 12) | Vilda (n = 10) | P-value |
|--------------------------------|------------------|----------------|---------|
| Body weight, g                 | 29.2 ± 2.2       | 35.7 ± 4.5     | 0.19    |
| Blood glucose (with fasting), mg/dL | 82.8 ± 5.7     | 96.0 ± 5.2     | 0.11    |
| Total cholesterol, mg/dL       | 705.1 ± 98.0    | 799.2 ± 100.1  | 0.51    |
| Triglyceride, mg/dL            | 68.8 ± 13.8      | 79.2 ± 14.1    | 0.61    |
| HDL-cholesterol, mg/dL         | 14.5 ± 2.0       | 21.7 ± 3.4     | 0.08    |
| Heart rate, bpm                | 605 ± 35         | 609 ± 45       | 0.95    |
| Systolic BP, mmHg              | 96.1 ± 3.2       | 94.6 ± 4.1     | 0.78    |
| Diastolic BP, mmHg             | 78.4 ± 1.9       | 76.9 ± 4.0     | 0.75    |
Figure 2. Effects of Vilda on the characteristics of atherosclerotic plaques. A: Oil red O staining demonstrated that Vilda reduced lipid deposition in atherosclerotic plaques in the aortic root compared with vehicle (n = 10-12, per group). B-D: Immunostaining against MOMA-2 (B), VCAM-1 (C), and ICAM-1 (D) demonstrated that Vilda reduced VCAM-1 expression and tended to decrease macrophage accumulation in atherosclerotic plaques in the aortic root compared with vehicle (n = 10-12, per group). Scale bar: 500 μm.

A crucial role in vascular homeostasis, whereas reduction of production and/or bioavailability of NO contributes to the development of atherosclerosis. NO is produced in the endothelium via nitric oxide synthase (NOS); atheroscle-
Figure 3. Vilda attenuated endothelial dysfunction in nondiabetic ApoE−/− mice. Vascular reactivity to Ach or SNP was determined using aortic rings isolated from Vilda- or vehicle-administered ApoE−/− mice and age- and sex-matched wild-type mice (n = 4-11, per group). ApoE−/− mice treated with vehicle showed impairment of endothelial function compared with wild-type mice. Vilda administration for 8 weeks ameliorated endothelium-dependent vasodilation in response to Ach compared with the vehicle group in ApoE−/− mice (A). Vasorelaxation in response to SNP did not differ among the three groups (B).

Table II. Effect of Vilda on Metabolic Parameters after 8-Week Treatment

|                      | Vehicle (n = 7) | Vilda (n = 11) | P-value |
|----------------------|----------------|---------------|---------|
| Body weight, g       | 23.7 ± 0.5     | 24.1 ± 0.6    | 0.72    |
| Blood glucose (without fasting), mg/dL | 134.1 ± 9.3 | 126.7 ± 5.5 | 0.47    |
| Total cholesterol, mg/dL | 1443.9 ± 73.3 | 1267.4 ± 63.5 | 0.09    |
| Triglyceride, mg/dL  | 78.6 ± 13.4    | 80.2 ± 10.3   | 0.93    |
| HDL-cholesterol, mg/dL | 16.0 ± 2.7    | 14.2 ± 1.5    | 0.53    |
| Heart rate, bpm      | 668.4 ± 29.6   | 661.1 ± 21.6  | 0.84    |
| Systolic BP, mmHg    | 109.7 ± 3.9    | 109.6 ± 4.3   | 0.98    |
| Diastolic BP, mmHg   | 67.7 ± 3.2     | 67.1 ± 2.6    | 0.88    |

Figure 4. Ex-4 attenuated endothelial dysfunction induced by LPS. To investigate the effects of Ex-4, a GLP-1 analog, on endothelial function, vascular reactivity to Ach or SNP was examined using aortic rings isolated from wild-type mice (n = 8, per group). Aortic rings were incubated with LPS (10 ng/mL) in the presence or absence of Ex-4 (10 nM) for 24 hours. Ex-4 significantly improved endothelium-dependent vasodilation which was impaired by LPS (A). Neither Ex-4 nor LPS had an effect on endothelium-independent vasodilation (B).
Coronary stimuli, such as hyperlipidemia and hyperglycemia, deteriorate this function. Recent studies have demonstrated that DPP-4 inhibitors increased NO production, leading to the improvement of endothelial function in human and animal studies. Furthermore, the results of a clinical study which investigated the effect of sitagliptin, a DPP-4 inhibitor, on endothelial function in coronary artery disease and uncontrolled diabetic patients suggested that it ameliorated endothelial dysfunction without blood glucose alteration. Also, previous studies have demonstrated that genetic deletion of eNOS by using eNOS-deficient mice or pharmacological blockade of eNOS by L-NG-nitroarginine methyl ester attenuated protective effects of DPP4 inhibitors on endothelial cells, including vascular relaxation and blood flow recovery. Therefore, the results of our present study suggested that Vilda improved endothelial cell function, especially in the early stages of atherosclerosis, by the activation of eNOS in nondiabetic animals independent of blood glucose.

In this study, we examined the effect of GLP-1 on endothelial function. Previous studies have demonstrated that GLP-1 has various protective effects on the endothelium. The results of our ex vivo experiments demonstrated that endothelial function was impaired by LPS in aortic segments isolated from wild-type mice, although Ex-4, a GLP-1 analog, ameliorated this response. Also, the results of our in vitro experiments using HUVEC demonstrated that Ex-4 ameliorated LPS-induced impairment of eNOS phosphorylation. Increased eNOS phosphorylation at Ser1177 suggests elevated NO production in endothelial cells.

In conclusion, Vilda attenuated endothelial dysfunction and reduced atherosclerotic lesions in nondiabetic ApoE−/− mice without an alteration of the blood glucose level. This study increases the understanding of the mechanisms by which Vilda attenuates atherosclerosis. Effects of DPP-4 inhibitors independent of glucose lowering may provide an attractive therapeutic option for atherosclerosis.

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Disclosure

Conflicts of interest: The Department of Cardio-Diabetes Medicine, Tokushima University Graduate School, is supported in part by unrestricted research grants from Boehringer Ingelheim. Dr. Sata received research funding from Novartis Pharma. Other authors declare that they have no conflicts of interest.

References

1. Anagnostis P, Adhyros VG, Adamidou F, et al. Glucagon-like peptide-1-based therapies and cardiovascular disease: looking beyond glycaemic control. Diabetes Obes Metab 2011; 13: 302-12.
2. Chrysant SG, Chrysant GS. Clinical implications of cardiovascular preventing pleiotropic effects of dipeptidyl peptidase-4 inhibitors. Am J Cardiol 2012; 109: 1681-5.
3. Ishizue N, Niwano S, Niwano H, et al. Linagliptin suppresses electrical and structural remodeling in the isoproterenol induced myocardial injury model. Int Heart J 2019; 60: 411-5.
4. Takahashi A, Ibata M, Yamazaki S, Asanuma H, Asakura M, Kitakaze M. Impact of either GLP-1 agonists or DPP-4 inhibitors on pathophysiology of heart failure. Int Heart J 2015; 56: 372-6.
5. Drucker DJ. Incretin action in the pancreas: potential promise, possible perils, and pathological pitfalls. Diabetes 2013; 62: 3316-23.

Figure 5. Ex-4 promoted phosphorylation of eNOS. To investigate the effect of Ex-4 on endothelial cells, phosphorylation of eNOSSer1177 was examined in HUVEC. Western blotting demonstrated that Ex-4 promoted phosphorylation of eNOSSer1177, which was impaired in the presence of LPS (n = 5, per group).
Husain M. Cardioprotective and vasodilatory actions of glucagon-like peptide 1 receptor are mediated through both glucagon-like peptide 1 receptor-dependent and -independent pathways. Circulation 2008; 117: 2340-50.

7. Ussher JR, Drucker DJ. Cardiovascular actions of incretin-based therapies. Circ Res 2014; 114: 1788-803.

8. Ervima N, Mita T, Yasunari E, et al. Anagliptin, a DPP-4 inhibitor, suppresses proliferation of vascular smooth muscles and monocyte inflammatory reaction and attenuates atherosclerosis in male apo E-deficient mice. Endocrinology 2013; 154: 1260-70.

9. Salim HM, Fukuda D, Higashikuni Y, et al. Dipeptidyl peptidase-4 inhibitor, linagliptin, ameliorates endothelial dysfunction and atherosclerosis in normoglycemic apolipoprotein-E deficient mice. Vascul Pharmacol 2016; 79: 16-23.

10. Shah Z, Kampfrath T, Deulius JA, et al. Long-term dipeptidyl-peptidase 4 inhibition reduces atherosclerosis and inflammation via effects on monocyte recruitment and chemotaxis. Circulation 2011; 124: 2338-49.

11. Terasaki M, Nagashima M, Nothorn K, et al. Preventive effect of dipeptidyl peptidase-4 inhibitor on atherosclerosis is mainly attributable to incretin’s actions in nondiabetic and diabetic apolipoprotein E-null mice. PLOS ONE 2013; 8: e70933.

12. Terasaki M, Nagashima M, Watanabe T, et al. Effects of PKF 275-055, a dipeptidyl peptidase-4 inhibitor, on the development of athero sclerotic lesions in apolipoprotein E-null mice. Metabolism 2012; 61: 974-7.

13. Matsubara J, Sugiyama S, Akiyama E, et al. Dipeptidyl peptidase-4 inhibitor, sitagliptin, improves endothelial dysfunction in association with its anti-inflammatory effects in patients with coronary artery disease and uncontrolled diabetes. Circ J 2013; 77: 1337-44.

14. Nakamura K, Oe H, Kihara H, et al. DPP-4 inhibitor and alpha glucosidase inhibitor equally improve endothelial function in patients with type 2 diabetes: EDGE study. Cardiovas Diabetol 2014; 13: 110.

15. Guerci B, Monnier L, Serusclat P, et al. Continuous glucose profiles with vildagliptin versus sitagliptin in add-on to metformin: results from the randomized Optima Study. Diabetes Metab 2012; 38: 359-66.

16. Sakamoto M, Nishimura R, Itoke T, Tsujino D, Ando K, Utsumoniya K. Comparison of vildagliptin twice daily vs. sitagliptin once daily using continuous glucose monitoring (CGM): crossover pilot study (J-VICTORIA study). Cardiovasc Diabetol 2014; 13: 110.

17. Ross R. Atherosclerosis— an inflammatory disease. N Engl J Med 1999; 340: 115-26.

18. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. Arterioscler Thromb Vasc Biol 2003; 23: 168-75.

19. Nemoto T, Minami Y, Yamaoka-Tojo M, et al. Impaired flow-mediated dilation and severity and vulnerability of culprit plaque in patients with coronary artery disease. Int Heart J 2019; 60: 539-45.

20. Higashi Y, Noma K, Yoshizumi M, Kihara Y. Endothelial function and oxidative stress in cardiovascular diseases. Circ J 2009; 73: 411-8.

21. Han T, Fukuda D, Tanaka K, et al. Inhibition of activated factor X by Rivaroxaban attenuates neointima formation after wire-mediated vascular injury. Eur J Pharmacol 2018; 820: 222-8.

22. Hara T, Fukuda D, Tanaka K, et al. Rivaroxaban, a novel oral anticoagulant, attenuates atherosclerotic plaque progression and destabilization in ApoE-deficient mice. Atherosclerosis 2015; 242: 639-46.

23. Gaspari T, Welungoda I, Widdop RE, Simpson RW, Dear AE. The GLP-1 receptor agonist liraglutide inhibits progression of vascular disease via effects on atherosclerosis, plaque stability and endothelial function in an ApoE(−/−) mouse model. Diab Vasc Dis Res 2013; 10: 353-60.

24. Salim HM, Fukuda D, Higashikuni Y, et al. Teneligliptin, a dipeptidyl peptidase-4 inhibitor, attenuated pro-inflammatory phenotype of perivascular adipose tissue and inhibited atherogenesis in normoglycemic apolipoprotein-E-deficient mice. Vascul Pharmacol 2017; 96-98: 19-25.

25. Michelsen KS, Wong MH, Shah PK, et al. Lack of toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. Proc Natl Acad Sci U S A 2004; 101: 10679-84.

26. Ta NN, Schuyler CA, Li Y, Lopes-Virella MF, Huang Y. DPP-4 (CD26) inhibitor alogliptin inhibits atherosclerosis in diabetic apolipoprotein E-deficient mice. J Cardiovasc Pharmacol 2011; 58: 157-66.

27. van Poppel NC, Netea MG, Smits P, Tack CJ. Vildagliptin improves endothelium-dependent vasodilatation in type 2 diabetes. Diabetes Care 2011; 34: 2072-7.

28. Dai Y, Wang X, Ding Z, Dai D, Mehta JL. DPP-4 inhibitors repress foam cell formation by inhibiting scavenger receptors through protein kinase C pathway. Acta Diabetol 2014; 51: 471-8.

29. Hirata Y, Kurobe H, Nishio C, et al. Exendin-4, a glucagon-like peptide-1 receptor agonist, attenuates neointimal hyperplasia after vascular injury. Eur J Pharmacol 2013; 699: 106-11.

30. Matheussens V, Vaumans Y, Martinet W, et al. Dipeptidyl peptidases in atherosclerosis: expression and role in macrophage differentiation, activation and apoptosis. Basic Res Cardiol 2013; 108: 350.

31. Vanhoucke PM. Endothelial dysfunction: the first step toward coronary atherosclerosis. Circ J 2009; 73: 595-601.

32. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation 2004; 109: III27-32.

33. Gimbrone MA Jr, García-Cardelás G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circ Res 2016; 118: 620-36.

34. Mason RP, Jacob RF, Kubant R, et al. Effect of enhanced glycemic control with saxagliptin on endothelial nitric oxide release and CD40 levels in obese rats. J Atheroscler Thromb 2011; 18: 774-83.

35. Ishii M, Shibata R, Kondo K, et al. Vildagliptin stimulates endothelial cell network formation and ischemia-induced revascularization via an endothelial nitric-oxide synthase-dependent mechanism. J Biol Chem 2014; 289: 27235-45.

36. Kim HJ, Baek EB, Kim SJ. Potentiation of endothelium-dependent vasorelaxation of mesenteric arteries from spontaneously hypertensive rats by gemigliptin, a dipeptidyl peptidase-4 inhibitor class of anti-diabetic drug. Korean J Physiol Pharmacol 2018; 22: 713-9.

37. Shah Z, Pineda C, Kampfrath T, et al. Acute DPP-4 inhibition modulates vascular tone through GLP-1 independent pathways. Vascul Pharmacol 2011; 55: 2-9.

38. Ceriello A, Novials A, Ortega E, et al. Glucagon-like peptide 1 reduces endothelial dysfunction, inflammation, and oxidative stress induced by both hyperglycemia and hypoglycemia in type 1 diabetes. Diabetes Care 2013; 36: 2346-50.

39. Erdogdu O, Eriksson L, Nyström T, Sjöholm Å, Zhang Q. Exendin-4 restores glucolipotoxicity-induced gene expression in human coronary artery endothelial cells. Biochem Biophys Res Commun 2012; 419: 790-5.

40. Wu L, Liu X, Wang L, et al. Exendin-4 protects HUVECs from tunicamycin-induced apoptosis via inhibiting the IRE1α/JNK/caspase-3 pathway. Endocrine 2017; 55: 764-72.

41. Colasanti M, Persichini T, Cavalleri E, et al. Rapid inactivation of NOS-I by lipopolysaccharide plus interferon-gamma-induced tyrosine phosphorylation. J Biol Chem 1999; 274: 9915-7.

42. Kamoun WS, Karas A, Kreese N, Merkel SM, Korneszczuk K, Clemens MG. LPS inhibits endothelin-1-induced endothelial NOS activation in hepatic sinusoidal cells through a negative feedback involving cavelon-1. Hepatology 2006; 43: 182-90.

43. Ding J, Song D, Ye X, Liu SF. A pivotal role of endothelial-specific NF-kappaB signaling in the pathogenesis of septic shock and septic vascular dysfunction. J Immunol 2009; 183: ...
4031-8.
44. Stark RJ, Koch SR, Choi H, et al. Endothelial nitric oxide synthase modulates toll-like receptor 4-mediated IL-6 production and permeability via nitric oxide-independent signaling. FASEB J 2018; 32: 945-56.
45. Erdogdu O, Eriksson L, Xu H, Sjöholm A, Zhang Q, Nyström T. Exendin-4 protects endothelial cells from lipoapoptosis by PKA, PI3K, eNOS, p38 MAPK, and JNK pathways. J Mol Endocrinol 2013; 50: 229-41.
46. Han L, Yu Y, Sun X, Wang B. Exendin-4 directly improves endothelial dysfunction in isolated aortas from obese rats through the cAMP or AMPK-eNOS pathways. Diabetes Res Clin Pract 2012; 97: 453-60.
47. Iwaya C, Nomiyama T, Komatsu S, et al. Exendin-4, a glucagonlike peptide-1 receptor agonist, attenuates breast cancer growth by inhibiting NF-κB activation. Endocrinology 2017; 158: 4218-32.
48. Krasner NM, Ido Y, Ruderman NB, Cacicedo JM. Glucagon-like peptide-1 (GLP-1) analog liraglutide inhibits endothelial cell inflammation through a calcium and AMPK dependent mechanism. PLOS ONE 2014; 9: e97554.
49. Steven S, Hausding M, Kröller-Schön S, et al. Gliptin and GLP-1 analog treatment improves survival and vascular inflammation/dysfunction in animals with lipopolysaccharide-induced endotoxemia. Basic Res Cardiol 2015; 110: 6.