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Dipeptidyl peptidase-4 inhibitor, linagliptin, ameliorates endothelial dysfunction and atherogenesis in normoglycemic apolipoprotein-E deficient mice

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ABSTRACT

Background: Dipeptidyl peptidase-4 (DPP-4) inhibitors have vasoprotective effects. This study investigated whether a recently approved DPP-4 inhibitor, linagliptin (Lina), suppresses atherogenesis in non-diabetic apolipoprotein-E deficient ($ApoE^{-/-}$) mice, and examined its effects on endothelial function.
Methods and results: Lina (10 mg/kg/day) was administered orally to $ApoE^{-/-}$ mice for 20 weeks. Lina reduced atherogenesis without alteration of metabolic parameters including blood glucose level compared with control ($P < 0.05$). Results of immunohistochemical analyses and quantitative RT-PCR demonstrated that Lina significantly decreased inflammatory molecule expression and macrophage infiltration in the atherosclerotic aorta. Lina administration to $ApoE^{-/-}$ mice for 9 weeks ameliorated endothelium-dependent vasodilation compared with that in untreated mice. Plasma active glucagon-like peptide-1 (GLP-1) level was significantly higher in the treated group ($P < 0.05$). Exendin-4 (Ex-4), a GLP-1 analog, ameliorated endothelium-dependent vasodilation impaired by palmitic acid (PA) in wild-type mouse aortic segments. Ex-4 promoted phosphorylation of $eNOS^{Ser1177}$ and Akt, both of which were abrogated by PA, in human umbilical vein endothelial cells. In addition, Lina administration to $ApoE^{-/-}$ mice decreased oxidative stress, as determined by urinary 8-OHdG secretion and NADPH oxidase subunit expression in the abdominal aorta.
Conclusion: Lina inhibited atherogenesis in non-diabetic $ApoE^{-/-}$ mice. Amelioration of endothelial dysfunction associated with a reduction of oxidative stress by GLP-1 contributes to the atheroprotective effects of Lina.

1. Introduction

Chronic inflammation plays a central role in the pathogenesis of atherosclerosis [1]. Endothelial dysfunction caused by cardiovascular risk factors is a key initiator of vascular inflammation [2]. Dysfunction of endothelial cells alters vascular responses and induces the expression of adhesion molecules and chemokines which stimulate monocyte-endothelial cell interaction, leading to the development of atherosclerosis [3]. Therefore, abrogation of endothelial dysfunction is an attractive strategy for preventing vascular inflammation and atherosclerosis.

Dipeptidyl peptidase-4 (DPP-4) inhibitors are a new class of antidiabetic drugs that improve glucose metabolism by raising the active concentration and duration of action of glucose-like peptide (GLP)-1, a gut hormone secreted in response to nutrient ingestion that stimulates glucose-dependent insulin secretion [4]. In addition to their antidiabetic property, recent studies have suggested that DPP-4 inhibitors have anti-inflammatory effects independent of their glucose-lowering effect [5–9]. The GLP-1 receptor mediates major function of GLP-1. The GLP-1 receptor is known to be expressed in pancreatic β cells, although recent studies demonstrated its expression in endothelial cells, suggesting vasoprotective effects independent of blood glucose level [10,11].

Abbreviations: DPP-4, dipeptidyl peptidase-4; Lina, linagliptin; $ApoE^{-/-}$, apolipoprotein-E deficient; GLP-1, glucagon-like peptide-1; Ex-4, exendin-4; PA, palmitic acid; WTD, western-type diet; CMC, carboxymethyl cellulose; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; MCP-1, monocyte chemoattractant protein-1; VCAM-1, vascular cellular adhesion molecule-1; Mac-3, macrophage antigen-3; Ach, acetylcholine; SNP, sodium nitroprusside; HUVEC, human umbilical vein endothelial cell; VEGF, vascular endothelial growth factor; qPCR, quantitative real-time PCR; ROS, reactive oxygen species.

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Table 1
Effects of Lina on metabolic parameters after 20-week treatment.

	Ctrl (N = 14)	Lina (N = 16)	P-value
Body weight, g	37.3 ± 2.0	34.2 ± 2.2	0.33
Blood glucose, mg/dL	92.9± 6.2	99.5± 6.0	0.45
Insulin, ng/mL	4.8± 0.7	4.2± 0.8	0.57
Total cholesterol, mg/dL	690.0 ± 49.8	554.6 ± 72.6	0.15
Triglyceride, mg/dL	73.5 ± 5.3	84.8 ± 10.8	0.39
HDL cholesterol, mg/dL	12.5 ± 1.3	11.5 ± 1.8	0.67
Heart rate, bpm	680.0 ± 20.8	687.7 ± 24.8	0.83
Systolic BP, mm Hg	97.7 ± 3.6	93.0 ± 1.5	0.19
Diastolic BP, mm Hg	74.1 ± 3.9	67.4 ± 2.1	0.12

BP; blood pressure, Ctrl; control, Lina; linagliptin. All values are mean ± SEM.

Previously, we have reported that the GLP-1 analog, exendin-4 (Ex-4), attenuated neointima formation after mechanical vascular injury by inhibiting macrophage activation at least partially in normoglycemic mice [12]. Also several studies have demonstrated that DPP-4 inhibitors suppressed the development of atherosclerosis even in non-diabetic atherosclerotic models [13–16]. However, the effects of DPP-4 inhibitors on endothelial cell function, associated with atherogenesis, in normoglycemic animals have not been fully investigated. Therefore, in this study, we administered linagliptin (Lina), which was recently approved as an oral glucose-lowering drug [17], to normoglycemic apolipoprotein E-deficient ($ApoE^{-/-}$) mice and examined the effects of Lina on endothelial cell function and atherogenesis. Our findings demonstrated that Lina reduced the development of atherosclerosis and ameliorated endothelial dysfunction in this mouse model. Results of in vitro and ex vivo experiments suggested that the antioxidant effect of GLP-1 contributed, at least partially, to these results.

2. Materials and methods

2.1. Animals and drug administration

$ApoE^{-/-}$ (C57BL/6J background) mice were originally purchased from The Jackson Laboratory. The $ApoE^{-/-}$ mouse, which exhibits severe hypercholesterolemia, is a widely used mouse model of atherosclerosis [18]. All experimental procedures conformed to the guidelines for animal experimentation of Tokushima University. Lina was supplied by Boehringer Ingelheim, Japan. To examine the effect of Lina on atherogenesis, 8-week-old male $ApoE^{-/-}$ mice receiving a western-type diet (WTD) were treated with Lina 10 mg/kg/day by gavage for 20 weeks. To examine the effect of Lina on endothelial function at earlier stage of atherosclerosis, the same dose of Lina was administered to 7-week-old female $ApoE^{-/-}$ for 9 weeks. WTD was started from 8 weeks of age. Lina was suspended in 0.5% carboxymethyl cellulose (CMC) solution. The control group received an equivalent volume of CMC. Mice were maintained under a 12 h light/dark cycle.

2.2. Blood pressure and laboratory data

The blood pressure of each mouse was measured by a tail-cuff system (BP-98A, Softron) as described previously. 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, HDL-cholesterol, and triglyceride levels were measured at LSI Medience Corporation (Japan). Plasma levels of insulin and active GLP-1 were measured using commercially available kits (Shibayagi Co., Ltd. and Immuno-Biological Laboratories Co., Ltd., respectively). Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) concentration in a 16 h urine collection was determined using a commercially available kit (Japan Institute for the Control of Aging, Nikken SEIL Co., Ltd.) and corrected by creatinine level.

2.3. Quantification of atherosclerotic lesions

The severity of atherosclerotic lesions in the aortas was assessed as previously described [19]. In brief, mice were sacrificed with an overdose of pentobarbital, and perfused with 0.9% sodium chloride solution at a constant pressure via the left ventricle. Both the heart and whole aorta were immediately removed. The thoracic aorta was excised, opened longitudinally, and fixed with 4% paraformaldehyde. To quantify atherosclerotic lesions in the aortic arch, we performed en face Sudan IV staining. The percentage of Sudan IV-positive area was measured. The abdominal aorta was removed and snap-frozen in liquid nitrogen for gene expression analysis.

2.4. Histological and immunohistochemical analyses

Frozen sections of the aortic root (at 5-μm intervals) were obtained as described previously [19]. The sections were stained with Oil red O to detect lipid deposition. Monocyte chemoattractant protein-1 (MCP-1), vascular cellular adhesion molecule-1 (VCAM-1), and macrophage antigen-3 (Mac-3) expression were detected using an anti-MCP-1 (BD Pharmingen), anti-VCAM-1 (Abcam) or anti-Mac3 (BD Biosciences) antibody followed by the avidin–biotin complex technique and stained using a Vector Red substrate kit (Vector). Each section was counterstained with hematoxylin.

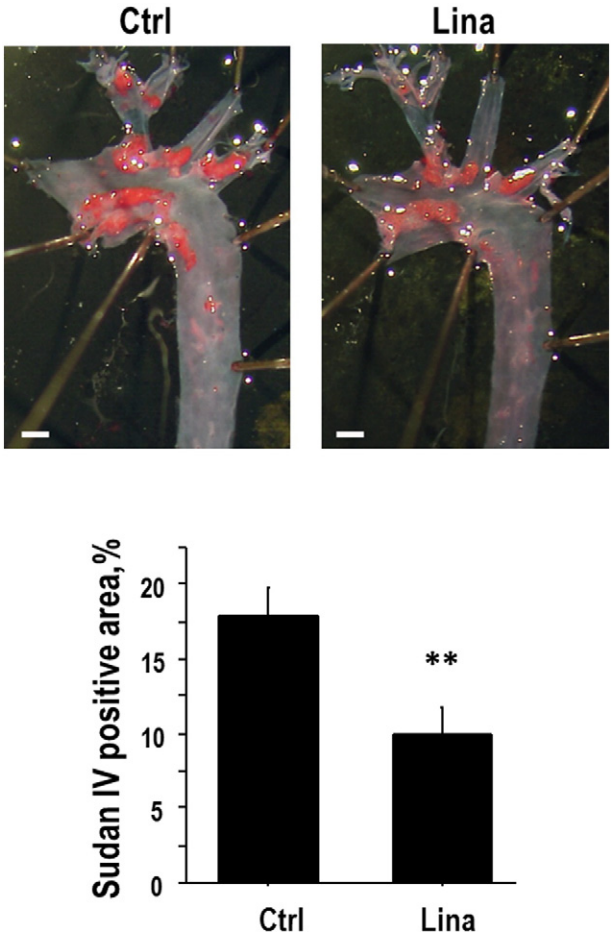

Fig. 1. Effect of Lina on atherosclerotic lesion progression in normoglycemic $ApoE^{-/-}$ mice. En face Sudan IV staining of the aortic arch showed that Lina administration for 20 weeks significantly reduced the progression of atherosclerotic lesions compared with vehicle (n = 12, per group). Bar: 1 mm. **; P < 0.01. All values are mean ± SEM.