Role of Dipeptidyl Peptidase-4 in Atherosclerotic Cardiovascular Disease in Humans and Animals with Chronic Stress

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Summary

Exposure to psychosocial stress is a risk factor for cardiovascular disease, including vascular atherosclerosis-based cardiovascular disease (ACVD). Dipeptidyl peptidase-4 (DPP-4) is a complex enzyme that acts as a membrane-anchored cell surface exopeptidase. DPP-4 is upregulated in metabolic and inflammatory cardiovascular disorders. DPP-4 exhibits many physiological and pharmacological functions by regulating its extremely abundant substrates, such as glucagon-like peptide-1 (GLP-1). Over the last 10 years, emerging data have demonstrated unexpected roles of DPP-4 in extracellular and intracellular signaling, immune activation, inflammation, oxidative stress production, cell apoptosis, insulin resistance, and lipid metabolism. This mini-review focuses on recent novel findings in this field, highlighting a DPP-4-mediated regulation of GLP-1-dependent and -independent signaling pathways as a potential therapeutic molecular target in treatments of chronic psychological stress-related ACVD in humans and animals.

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Key words: Vascular senescence, Atherosclerosis, Inflammation, Oxidative stress

Chronic psychological stress (CPS) is considered a risk factor for vascular aging and atherosclerosis-based cardiovascular disease (ACVD), based on clinical and experimental observations (Table).¹⁾ The importance of various psychological stressors as contributors to the initiation and progression of vascular senescence and ACVD has been the focus of concerted research efforts over the past several decades.²⁻⁵⁾ For example, the large-scale case-control INTERHEART trial conducted in 51 countries demonstrated that chronic psychological stressors (e.g., depression, perceived life stress, major life events, and low sense of control) pose an adjusted 2.7-fold enhanced risk of acute myocardial infarction (AMI).⁶⁾ Indeed, the contribution of psychological factors (e.g., anxiety and depression) to the increased likelihood of recurrent coronary arterial events after coronary artery bypass grafting and AMI is known, and it is well documented that transient psychological stress may cause potentially fatal arrhythmias and acute cardiovascular events.⁷⁾ Over the last 10 years, it has been established that chronic psychological stressors in modern lifestyles are associated closely with the incidence of hypertension, metabolic syndrome, diabetes mellitus (DM), and cardiovascular diseases (CVDs).⁸⁾ Clinical and laboratory findings from our team and other groups showed that chronic

psychological stressors activate intra- and extracellular pathways (including the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system) by eliciting pathophysiological overactions, resulting in metabolic and inflammatory cardiovascular disorders (Table).⁹⁻¹²⁾ However, the precise mechanisms involved in stress-related vascular aging and atherosclerotic lesion formation and progression remain largely uncertain.

A Brief Review of DPP-4

The biological and molecular functions of DPP-4: The human gene encoding dipeptidyl peptidase-4 (DPP-4) has been reported to localize to chromosome 2 locus 2q24.2.¹³⁾ DPP-4 is a member of a complex gene family, several members of which nonspecifically truncate many structure-related peptides, including cytokines, chemokines, neuropeptides, hormones, and growth factors.¹⁴⁾ Figure 1 shows the gene family of DPP-4-related proteases and their expression of cell types, substrate specificities, working spaces, and functions. The DPP-4 protease family includes *N*-acetylated- α -linked acidic dipeptidase I, II, L, seprase, DPP-1v β , DPP-6, DPP-8, DPP-9, fibroblast activation protein α , folate hydrolase, prostate-specific membrane antigen, pteroylpoly- γ -glutamate carboxypeptidase,

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Table. Experimental Studies of Chronic Stressors on Vascular Aging, Angiogenesis, and Atherosclerosis

Diseases and implications	Animals Publication year/journal	Stressor	Treatment	Mechanism	Morphological and functional alterations	Ref.
Angiogenesis	BALB/c (2015)	Immobilized stress (3 weeks)	Fluoxetine hydrochloride (18 mg/kg/day)	Oxidative stress ↓ / VEGF ↑ / p-Erk1/2 ↑	Blood flow ↑ Capillary density ↑	5
Metabolic disorder Prothrombosis	C57BL/6j (2012)	Immobilized stress (2 weeks)	7ND Human MCP-1 neutralizing antibody	Inflammation ↓ GTT (–) /ITT ↓ PAI-1/TF ↓ MCP-1 ↓ TNF-α/IL-6 ↓	Macrophage infiltration ↓ Insulin resistance ↓ Prothrombosis ↓	21
HSC activation Atherosclerosis	C57BL/6J, ApoE ^{–/–} (B6.129P2-Apoetm1Unc/J), (2014) Nature Medicine	Chronic variable stress (6 weeks)		Bone marrow niche Adrβ3 ↑ /CXCL12 ↓ Plasma adrenaline/ Noradrenaline ↑	Bone marrow lin [–] c-Kit ^{high} Sca-1 ^{high} CD48 ^{low} CD150 ^{high} HSCs ↓ Peripheral blood neutrophils ↓ Monocytes ↓ Leukocytes ↓ Atherosclerotic plaque ↑	25
HSC activation	C57BL/6j DPP4 ^{–/–} rats (2017) Journal of American Heart Association	Immobilized stress (2 weeks)	Anagliptin (30 mg/kg/day) Exenatide (5 μg/kg/day) A specific Adrβ3 inhibitor (L748337: 0.5 mg/kg/day)	Brain GLP-1R ↑ Bone marrow niche Adrβ3 ↓ /CXCL12 ↑ Plasma adrenaline/ Noradrenaline ↓ MMP-2/MMP-9 ↓ Plasma DPP4 ↓ /APN ↑	Bone marrow lin [–] c-Kit ^{high} Sca-1 ^{high} CD48 ^{low} CD150 ^{high} HSCs ↓ Peripheral blood neutrophils ↓ Monocytes ↓ Leukocytes ↓	26
Metabolic disorder Prothrombosis	C57BL/6j (2012) Plos One	Immobilized stress (2 weeks)	AT1R Antagonist (Irbesartan: 3 or 10 mg/kg/day)	Inflammation ↓ Angiotensinogen ↓ GTT (–) /ITT ↓ PAI-1/TF ↓ MCP-1 ↓ TNF-α/IL-6 ↓ Free fatty acid ↓ GLUT4/IRS-1 ↑ APN ↑	Macrophage infiltration ↓ Insulin resistance ↓	27
Metabolic disorder Prothrombosis	C57BL/6j (2016) Psychoneuroendocrinology	Immobilized stress (2 weeks)	DPP4 inhibitor (alogliptin: 15 or 45 mg/kg/day)	Macrophages ↓ GTT/ITT ↓ 8-OHdG ↓ Nox4/MCP-1 ↓ PAI-1/TF ↓ TNF-α/IL-6 ↓ GLUT4/IRS-1 ↑ Plasma DPP4 ↓ APN/GLP-1 ↑	Macrophage infiltration ↓ Insulin resistance ↓ Prothrombotic state ↓	29
Metabolic disorder Hyperuricemia	C57BL/6j (2017) Scientific Reports	Immobilized stress (2 weeks)	OX inhibitor (febuxostat: 1 or 5 mg/kg/day)	Plasma and tissue OX/MDA/XOR activity ↓ NADPH oxidase subunit mRNAs ↓ ROS production ↓ Mn-SOD mRNA ↑ Catalase mRNA ↑ Macrophages ↓ GTT/ITT ↓ MCP-1 ↓ PAI-1/TF ↓ TNF-α/IL-6 ↓ GLUT4/IRS-1 ↑	Macrophage infiltration ↓ Hyperuricemia ↓ Glucose Dysmetabolism ↓ Prothrombotic state ↓	30
Vascular aging Atherosclerosis	ApoE ^{–/–} mice (KOR/Stm Slc-ApoE ^{sh} , BALB/c background) (2017) International Journal of Cardiology	Chronic variable stress (12 weeks)	DPP4 inhibitor (anagliptin: 30 mg/kg/day) APN neutralizing antibody (450 μg/kg/day)	Plasma DPP4 ↓ APN/GLP-1 ↑ TLR2-/TLR-4 ↓ CXCR4/MCP-1 ↓ NADPH oxidase subunit ↓ MMP-2/MMP-9 ↓ TIMP-1/TIMP-2 ↓ CatS/CatL/CatK ↓	Vascular senescence ↓ Neovessel ↓ Lipid accumulation ↓ Collagen content ↑ Elastin broken ↓ SMC contents (–)	31

Table. Experimental Studies of Chronic Stressors on Vascular Aging, Angiogenesis, and Atherosclerosis (continued)

Diseases and implications	Animals Publication year/journal	Stressor	Treatment	Mechanism	Morphological and functional alterations	Ref.
Vascular aging Angiogenesis Neovascularization	C57BL/6j DPP4 ^{-/-} rats APN ^{-/-} mice (2017) Journal of American Heart Association	Chronic variable stress (4 weeks)	Anagliptin (low: 30 mg/kg/ day; high: 60 mg/ kg/day) Exenatide (5 µg/kg/day) APN neutralizing antibody (450 mg/kg/day)	Plasma DPP4 ↓ Corticosterone ↑ GLP-1/APN ↑ p-APMKα/Sirt-1 ↑ PPAR-γ/PGC-1α ↑ VEGF ↑ MMP-2/MMP-9 ↓ GLUT4/IRS-1 ↑ Macrophages ↓	Blood flow ↑ Capillary density ↑ Amputation ↑ Inflammation ↓	40
Vascular aging	C57BL/6j (2019) Chemico- Biological Interactions	Chronic immobilized stress (2 weeks)	Anagliptin (30 mg/kg/day)	Plasma DPP4 ↓ eNOS/Sirt1 ↑ p53/p21/p27 ↓ gp91 ^{phox} /p22 ^{phox} ↓ MMP-2/MMP-9 ↓ CatS/CatL/CatK ↓	Vascular aging ↓	41
Vascular aging Atherosclerosis	ApoE ^{-/-} mice (KOR/Stm Slc-ApoE ^{sh} , BALB/c back- ground) (2017) Atherosclerosis	Chronic variable stress (12 weeks)	GLP-1 analogue (exenatide: 5 µg/kg/day)	Plasma APN/leptin ↑ eNOS ↑ TLR2-/TLR-4 ↓ CXCR4/SDF-1 ↓ gp91 ^{phox} /p22 ^{phox} ↓ MMP-2/MMP-9 ↓ TIMP-1/TIMP-2 ↓ CatS/CatL/CatK ↓	Vascular senescence ↓ Neovessel ↓ Lipid accumulation ↓ Collagen content ↑ Elastin broken ↓ SMC contents (-)	42

7ND indicates dominant negative mutation of monocyte chemoattractant protein-1; MCP-1, monocyte chemoattractant protein-1; HSC, hematopoietic stem cell; Adrβ3, β3 adrenaline receptor; AT1R, angiotensin II type 1 receptor; VEGF, vascular endothelial growth factor; p-Erk1/2, phosphate-extracellular signal regulated kinase-1/-2; OX, xanthine oxidase; GTT, glucose tolerance test; ITT, insulin tolerance test; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; PAI-1, plasminogen activator-1; TF, tissue factor; GLUT4, glucose transporter type 4; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; GLP-1, glucagon-like peptide-1; DPP-4, dipeptidyl peptidase-4; APN, adiponectin; MDA, lipid peroxidation; NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species; XOR, xanthine oxidoreductase; Mn-SOD, Mn-superoxide dismutase; SMCs, smooth muscle cells; CatS, cathepsin S; MMP-2, matrix metalloproteinase-2; CXCR4, C-X-C chemokine receptor type 4; SDF-1, stromal derived factor-1; eNOS, endothelial nitric oxide synthase; p-AMPKα, phospho-AMP-activated protein kinase α; PPAR-γ, peroxisome proliferator-activated receptor-γ; PGC-1α, PPAR-γ co-activator; DPP4^{-/-}, DPP4 deficiency; ApoE^{-/-}, apolipoprotein deficiency; APN^{-/-}, adiponectin deficiency; Ref., reference; (-), no change; ↑, increase; and ↓, decrease or improvement.

quiescent cell proline dipeptidase, thymus-specific serine protease, attractin, and other DPP-4 activity and/or structural homologues.¹⁵⁾ The adenosine deaminase immunoaffinity chromatography assay showed that soluble CD26/DPP-4 was responsible for the release of X-pro dipeptides.¹⁶⁾ Accumulating evidence suggests that the assessment of selectivity of potential clinical candidates may be important to create an optimal safety profile for this new class of antihyperglycemic agents.^{17,18)}

DPP-4 is a widely expressed glycoprotein that has attracted much attention both as a receptor for the Middle Eastern respiratory syndrome virus and for its role in the proteolytic degradation of gastrointestinal hormones, such as glucose-dependent insulintropic polypeptide and glucagon-like peptide-1 (GLP-1).¹⁴⁾ DPP-4 consists of three structural domains: a transmembrane α-helix domain, a C-terminal extracellular domain, and an N-terminal intracellular domain.¹⁹⁾ Iwaki-Egawa and colleagues reported that the N-terminal intracellular domain contributes to the enzymatic activity,²⁰⁾ and it was later demonstrated that the transmembrane region is responsible for the main enzymatic activity.¹⁹⁾ In addition to the transmembrane domain, DPP-4 presents as a soluble form, which is the extracellular domain of the peptide considered to be cleaved from the cell plasma membrane.¹⁹⁾

Since being identified, DPP-4—also known as T-cell activation antigen CD26 or Adenosine deaminase-binding protein 2—was known to be a 766-amino acid serine exopeptidase belonging to the S9B protein family and to degrade two alanine or X-proline dipeptides from the N-terminus of polypeptides in the extracellular space.²¹⁾ DPP-4 has been reported to be widely expressed on cell surface peptidase, which has a complex biology, participating in the cell membrane-related activation of cell-cell cross-talk, intracellular signal transductions, and the proteolytic activity displayed by the membrane-anchored and soluble forms of the enzyme.^{22,23)} Over the last 10 years, emerging findings demonstrated unexpected roles of DPP-4 in intracellular signaling, oxidative stress production, lipid metabolism, insulin resistance, immune activation, and inflammation.^{24,25)} These activities provide a broad range of molecular functions of the DPP-4 family (Figure 1), with clinical implications for a potential pathophysiological role in metabolic and inflammatory CVDs. Recent data from laboratory and clinical studies highlighted the role of DPP family members (especially DPP-4) in ACVD.²⁶⁻²⁸⁾

Cell and tissue expression of DPP-4: DPP-4, which is one of the most potent serine peptidases, is broadly expressed in mammalian tissues, including the kidney, blood vessels, small intestine, lung, liver, brain, spleen, adipose,

		<u>Specificities</u>	<u>Cell Types</u>	<u>Intra-/extracellular</u>	<u>Functions</u>
Related proline-specific peptidase	Prolidase	NH2-Xaa~Pro-COOH	Cancer cells	(+)/(-)	Proline metabolism
	APP	NH2-Xaa~Pro-Yaa--	T cells Cancer cells	(+)/(-)	Metal ion binding
	QPP/DPP-II	NH2-Xaa~Pro-Yaa--	Quiescent lymphocytes	(+)/(-)	Physiological substrates unknown T cells apoptosis
DPP-IV gene family	PREP	--Xaa-Pro~Yaa--	Tumor cells		Male Reproductive Functions/Gamete
	DPP-6	Proteolytically inactive	Cerebellar granule cells	(+)/(-)	Unknown
			P19 cells		Neural disease?
	FAP- α		Stromal fibroblasts	?/(+)	Like collagenase/ Collagen turnover Tumor growth/invasion
	DPP-4	NH2-Xaa~Pro-Yaa--	Immune cells/ECs Inflammatory cells	(+)/(+)	Many substrates (see Fig.3)
	DPP-8		Immune cells Epithelia	(+)/(-)	Various cellular functions Glucose homeostasis
	DPP-9		Muscle Brain cells Purkinje cells Acinar cells	(+)/(-)	Physiological substrates Unknown, but it cleaves GLP-1/GLP-2/PYY Various cellular functions

Figure 1. The substrate specificities, cell expression, working spaces, and functions of the family of DPP-4-related enzymes. ECs indicates endothelial cells; FAP- α , fibroblast activation protein- α ; APP, aminopeptidase P; QPP, quiescent cell praline dipeptidase; PREP, prolyl endopeptidase; GLP, glucagon-like peptide; PYY, peptide YY; (+), working in intra- or extracellular space; (-), not working in intra- or extracellular space; and ?, unknown.

and heart tissues (Figure 2).¹⁰⁾ The observations of an early experimental study using colorimetric enzyme histochemistry demonstrated that DPP-4 activity is localized in cardiac venous capillaries. DPP-4 is also expressed on endothelial progenitor cells, endothelial cells, some important immune cells (e.g., monocytes, dendritic cells, natural killer cells, and lymphocytes) and inflammatory cells (i.e., macrophages) in various pathological conditions.^{14,19)} As it is distributed widely, DPP-4 inhibition is a promising approach in various medical fields, such as inflammation regulation, hematopoiesis recovery, and immunomodulation, and, of course, in vascular repair and ischemic ACVD,^{27,29,30)} as highlighted in this review.

DPP-4 Substrates

Numerous neuropeptides, hormones, chemokines, growth factors, and endocrines contain an alanine or proline at position 2 and are putative DPP-4 substrates. DPP-4 exhibits many physiological and pharmacological functions by truncating extremely abundant substrates.¹⁹⁾ It is well known that DPP-4 inhibition can enhance insulin secretion and improve glucose tolerance in humans via the GLP-1-dependent signaling pathway.³¹⁾ Synthetic DPP-4 inhibitors were also reported to ameliorate glucose intolerance in *Glp1r*^{-/-} mice,³²⁾ suggesting that DPP-4 exerts its own biological role, independent of GLP-1. In addition,

DPP-4 degrades a large number of peptide chemokines and hormones *in vitro*, whereas comparatively limited peptides have been characterized as endogenous physiological substrates for DPP-4 *in vivo*.¹⁴⁾ Based on DPP-4's potential catalytic activity *in vivo and in vitro*, it seems that the proteins/peptides with cleavage sites for DPP-4 could be potential substrates of DPP-4 in various pathophysiological conditions.

Accumulating evidence indicates putative praline or N-terminal alanine DPP-4 truncation sites in many chemokines, cytokines, growth factors, and hormones, for example, interleukin-3 (IL-3), IL-1 α , IL-6, colony-stimulating factor (CSF), stromal cell-derived factor-1 α , granulocyte-CSF, granulocyte macrophage-CSF, erythropoietin, a number of splice variants of vascular endothelial growth factor-A, leukemia inhibitory factor, thrombopoietin, high-mobility group box 1, and others.¹⁹⁾ This raises the possibility that DPP-4 can modulate ACVD initiation and progression through the degradation and modification of these substrate-related factors. Given the potential effects of DPP-4 on ACVD, DPP-4 inhibitors have recently been known as pharmacological targets for ischemic ACVD. In the following sections, we focus on the significance of DPP-4/its inhibitor-mediated cardiovascular benefits on vascular inflammatory and metabolic CVDs in animals and humans under CPS.

Figure 2. Dipeptidyl peptidase-4 (DPP-4) is known to be expressed widely in human and animal tissues (e.g., vessels, blood, heart, small intestine, lung, kidney, brain, spleen, muscle, and visceral fat) (A) and vascular cells and ischemic cardiovascular disease (ICVD) -associated cells (B). EPCs indicates endothelial progenitor cells; NKCs, natural killer cells; TCs, T cells; Tm/Th1, T-memory/T-helper; DC, dendritic cells; (+), increase in diseased tissues; (±), increase/no changes in diseased tissue; and (-), no detection.

The Impact of CPS on ACVD and Its Mechanisms

Stress produces plasma and tissue DPP-4 and GLP-1 imbalance: Individual DPP family members may participate in inflammatory and metabolic disorders.^{33,34} The importance of DPP-4 in the initiation and progression of ACVD and the data on DPP-4 inhibition-mediated beneficial effects obtained from experimental models, mechanistic human studies, and clinical trials are summarized in two early comprehensive reviews.^{19,31} The discovery of incretin-based treatments exhibits a major therapeutic advance in the medical intervention of cardiometabolic disorders, and the development of DPP-4 inhibitors as useful antidiabetic drugs was based on the concept that these agents would enhance systematic and tissue glucagon-like peptide-1 (GLP-1) levels, causing an improvement of the insulinotropic effects of blood sugar.^{35,36} In addition to GLP-1-dependent effects on the cardiometabolic risk profile, DPP-4 inhibitors provide vascular protective beneficial effects by modulating several substrate factor activities (e.g., CSF, stromal cell-derived factor-1 α , granulocyte-CSF, granulocyte macrophage-CSF, neuropeptide Y, and high-mobility group box 1).³⁷ A clinical study reported that individuals with and without DM had increased plasma DPP-4 levels and decreased plasma GLP-1 levels.³⁸ In mice and rats, chronic stress increased circulating and tissue DPP-4 activities and decreased plasma and brain GLP-1 levels,^{2,26,27} suggesting an imbalance between GLP-1 and DPP-4 as a potential therapeutic target in the management of vascular aging and atherosclerosis in animals under experimental stress conditions.

DPP-4 inhibition attenuated vascular aging and atherosclerosis via the reduction of inflammation and oxidative stress production associated with GLP-1-mediated adiponectin production in response to stress:

Although a growing body of evidence indicates that DPP-4 plays an important role in the initiation and progression of ACVD,¹⁹ little is known about the functional relevance of this exopeptidase as a transmembrane protease in the pathogenesis of stress-related vascular senescence and atherogenesis. Chronic variable stress has been exhibited to produce harmful changes in blood and tissue DPP-4 levels.^{10,39} It is well known that inflammation occurs in all stages of atherosclerosis, including initiation, progression, calcification, plaque rupture, and ultimately, thrombotic complications.⁴⁰ Data from our research team and those from other groups clearly revealed that chronic variable stress activated bone-marrow hematopoietic stem cell proliferation via the inactivation of β -adrenergic receptor-mediated C-X-C motif chemokine 12 (CXCL12) (Table), leading to an increased output of inflammatory monocytes and neutrophils (Figure 3).^{9,10} Existing evidence has confirmed that stress can increase inflammatory actions in vascular and adipose tissues.^{2,4} *In vivo*, marked increases in neutrophil and macrophage infiltration and inflammatory chemokine/cytokine expressions (i.e., monocyte chemoattractant protein-1, osteopontin, toll-like receptor, and CXCR4) and vascular aging were observed in the aortas of stressed mice, and these changes were rectified significantly by DPP-4 inhibitor anagliptin treatment (Figure 3).²⁷

Accumulating evidence suggests that oxidative stress also plays a critical role in vascular senescence and atherosclerotic plaques in animals and humans.⁴¹ The observa-

Figure 3. The proposed mechanisms of how GLP-1R activation and DPP-4 inhibition suppress stress-related vascular endothelial senescence and atherosclerotic lesion formation in mice fed a high-fat diet. Stress enhanced the levels of blood DPP-4 levels and decreased blood GLP-1, which decreased adipose APN expression and promoted atherosclerotic lesion oxidative stress production, inflammation, and proteolysis, leading to an acceleration of vascular senescence and atherosclerotic lesion formation and its instability in ApoE^{-/-} mice. CPS indicates chronic psychological stress; DPP-4, dipeptidyl peptidase-4; APN, adiponectin; PPAR-γ, peroxisome proliferator-activated receptor-γ; Adrβ3, β3 adrenaline receptor; GLP-1R, GLP-1 receptor; PGC-1α, PPAR-γ co-activator-1α; GLP-1, glucagon-like peptide-1; SMCs, smooth muscle cells; ECs, endothelial cells; Mac, macrophages; HSC, hematopoietic stem cell; MMP-2, matrix metalloproteinase-2; and CatS, cathepsin S.

tions described herein exhibit that anagliptin mitigated NADPH oxidase component expression (p22phox, p47 phox, p67phox, and gp91phox) and superoxide (O₂⁻) generation. Moreover, the levels of the adiponectin protein and gene were increased in the blood, inguinal, and subcutaneous adipose tissues of stressed apoE lipoprotein-deficient (ApoE^{-/-}) mice, and these changes were reversed by DPP-4 inhibition.²⁷⁾ *In vitro*, the GLP-1 analog exenatide increased adiponectin expression in adipose tissue-derived immature adipocytes in a dose-dependent manner, whereas anagliptin did not affect it.²⁷⁾ Surprisingly, adiponectin depletion with its neutralizing antibody almost completely diminished the anagliptin-mediated vascular benefits in ApoE^{-/-} mice fed a high-fat diet.²⁷⁾ Adiponectin was protective against various vascular injuries under conditions of stress.⁴²⁾ These findings thus indicate that an enhancement of GLP-1 by DPP-4 inhibition may have provided a positive modulation of vascular senescence and atherosclerotic lesion formation through the improvement of adiponectin-induced antioxidative stress production and anti-inflammation in ApoE^{-/-} mice under our experimental conditions (Figure 3). This notion was further supported

by the findings of a comparable effect of exenatide on stress-related vascular harmful changes in ApoE^{-/-} mice fed a high fat-diet.³⁹⁾

DPP-4 inhibition prevents stress-related atherosclerotic plaque growth via the reduction of proteolysis: Accumulating evidence of vascular cells have reported that atherosclerosis-associated inflammatory cytokines augment the expression and production of the members of cathepsin and matrix metalloproteinase (MMP) families from cultured vascular cells (i.e., vascular smooth muscles and endothelial cells), monocyte-derived macrophages, mast cells, and T lymphocytes, and that these inflammatory cytokines increase the degradation of extracellular matrix proteins (collagen and elastin).⁴³⁾ Novel insights into the actions of these proteases have been made possible by the generation and in-depth analyses of transgenic and knockout mice.⁴⁴⁾ It is well known that both cathepsin and MMP activities modulate neovascularization and vascular remodeling through the modification, activation, and liberation of cytokines, angiogenic growth factors, cell events (apoptosis, transmigration, and proliferation), neovascularization, and matrix protein metabolism.⁴³⁾ Pharmacological

inhibitors targeting GLP-1 receptor stimulation and DPP-4 activity exhibited a protective effect on the expression and/or activities of proteolytic enzymes [e.g., cathepsin L (CatL), CatS, CatK, matrix metalloproteinase-2 (MMP-2), and MMP-9] and matrix protein metabolism (elastin and collagen) in the lesions of stressed animals fed a high-fat diet.^{2,27,39)} These therapies also suppressed the levels of plaque peroxisome proliferator-activated receptor- α (PPAR- α) and angiotensin II type 1 receptor (AT1R) proteins.²⁷⁾ Both receptor systems with their ligands have been exhibited to regulate CatS/K and MMP-2/-9 expression by the enhanced productions of oxidative stress and inflammatory cytokines both *in vivo* and *in vitro*.⁴³⁾ *In vitro*, exenatide suppressed tumor necrosis factor- α expression in cultured macrophages. These findings thus suggest that atherosclerotic lesion development with neovascularization and instability may be attributable to the increase in MMP-2/-9- and CatL/S/K-mediated proteolysis induced by the stimulation of PPAR- α - and AT1R-signaling pathway-related oxidative stress production and inflammation in animals under chronic stress conditions (Figure 3).

DPP-4 inhibition and GLP-1 receptor activation attenuated atherosclerosis via the modification of lipid metabolism: Previous clinical and basic research studies indicated that GLP-1 and DPP-4 activities are involved in lipid metabolism.^{27,31,39)} Biological analyses demonstrated that anagliptin reduced blood nonesterified fatty acids and triglycerides in stressed animals,²⁷⁾ and similar results were found in stressed animals treated with exenatide.³⁹⁾ Exenatide dramatically reduced the foam cell formation of peripheral blood monocyte-derived macrophages.³⁹⁾ Clinical observations have provided a limited beneficial effect of DPP-4 inhibition on plasma apolipoprotein B-48 and triglyceride levels.³¹⁾ Therefore, the improvements in free fatty acid and triglyceride metabolism may also contribute to the incretin-based glucose-lowering drug-related vascular benefits in mice under stress. Unfortunately, we observed that these treatments did not alter plasma levels of “good” and “bad” cholesterol (i.e., high- and low-density lipoprotein cholesterol) in animals under our experimental stress conditions.^{27,39)} However, clinical research provides evidence that these preclinical observations are translated into clinical practice.⁴⁵⁾

Clinical Trials with DPP-4 Inhibitors in CVD

Incretin-based DPP-4 inhibitors are often the chosen antidiabetic medications that are now most widely used globally. The outcomes of several clinical trials to evaluate the cardiovascular safety of DPP-4 inhibitors have been reported.⁴⁶⁻⁴⁹⁾ SAVOR-TIMI53 was designed as a superiority trial and failed to meet the prespecified superior outcomes of saxagliptin versus placebo in a high-risk patient population with established vascular diseases and risk factors.⁴⁶⁾ In the EXAMINE trial, designed as a safety trial in a high-risk population with post-acute coronary syndrome, the prespecified end point of non-inferiority was met, and alogliptin was non-inferior to the placebo with regard to cardiovascular outcomes.⁴⁷⁾ Despite the many preclinical studies showing the beneficial effects of incretin-related

drugs, most cardiovascular safety trials of incretin-based DPP-4 inhibitors did not show benefits for cardiovascular events. It is important to recognize that cardiovascular safety trials were carried out to meet the US Food and Drug Administration guidance to assess cardiovascular safety of all new antidiabetic drugs; they were not designed to assess their benefits for cardiovascular events. Therefore, the long-term potential benefits, as well as the safety, of DPP-4 inhibitors for certain cardiovascular outcomes have not been definitively established, and must be evaluated in more specific and relevant trials. If the need for cardiovascular safety trials is determined based on an individual drug's safety data during its early development as well as its mechanism of action, resources could be saved when carrying out such clinical trials.

In addition, these negative clinical findings are inconsistent with the positive results of animal studies,^{10,12,26,27)} which demonstrated a DPP-4 inhibitor-mediated cardiovascular protective effect in animal studies. Although based on their exclusion and inclusion criteria, these large-scale clinical trials recruited huge numbers of participants with different conditions (e.g., age, sex, body mass index, medical and medication histories, etc.), especially, ACVD risk factors and its complications.⁴⁶⁻⁴⁹⁾ In contrast, in animal studies, DPP-4 inhibitors were applied to investigate whether they produce a cardiovascular beneficial effect in various special simplified animal models (e.g., an ischemia-induced hindlimb model, a carotid artery ligation with and without cuff-replacement model, a myocardial infarction model, or a diet-induced atherosclerosis model) or model mice (ApoE^{-/-} mice, etc.).¹⁴⁾ With the exception of the vehicle and drugs used, the animal conditions (e.g., age, sex, body weight, genetic background, etc.) were controlled to be the same or similar between experimental groups. These factors possibly explain the inconsistent DPP-4 inhibitor-mediated cardiovascular effects between humans and animals. Further studies will be needed to investigate this issue.

Plasma DPP-4, GLP-1, and Adiponectin Levels as Novel Biomarkers for Chronic Stress and Related CVD Risk

Recently, it was reported that increased circulating DPP-4 and decreased circulating adiponectin and GLP-1 might serve as new useful biomarkers to predict the presence of stress in animals.^{10,26)} The observations suggest that among these biological parameters, blood DPP-4 levels were more sensitive to chronic stress, and that the noninvasive evaluation of those alterations would be useful to assess brain injuries in animals subjected to chronic stress.¹⁰⁾ However, the clinical significance of targeted hormone and exopeptidase changes in the initiation and progression of ACVD associated with modern stressors in humans (including natural disasters, environmental stress, work-related stress, and social anxiety) should be studied as large-scale prospective and/or retrospective cohort studies.

Concluding Remarks

Overall, recent findings from Lei, *et al.* suggest a potential clinically applicable benefit of DPP-4 inhibitors on vascular senescence and atherogenesis in ApoE^{-/-} mice under experimental stress conditions,²⁷⁾ with an effect size similar to that of GLP-1 analog-based therapies.³⁹⁾ This protective effect is supported by the finding of a consistent effect of genetic inhibition targeting DPP-4 on inflammation cell production and cytokine expression in the peripheral blood of rats under chronic stress.¹⁰⁾ These results are consistent with the positive findings of a small clinical trial of a GLP-1 analog or DPP-4 inhibitor as a useful treatment to mitigate atherosclerotic lesion formation and coronary artery events in patients with ACVD with and without DM.^{36,50)} Nevertheless, given the safety profile of DPP-4 inhibitors and most current, near-term clinical trial data, these intriguing data make a compelling case for long-term observational studies of GLP-1 analogs and DPP-4 inhibitors on CPS-related ACVD. There are some limitations to these current observations as follows: (1) The absence of preclinical dose dependence when comparing dose administration at 30, 60, and 90 mg/kg/day; (2) the use of DPP-4 inhibitors in T1DM and T2DM under physiological or psychological stress has not been studied rigorously in long-term, randomized clinical trials; (3) cardiovascular safety endpoints (e.g., chronic heart failure, hospitalization, sudden death) have been studied rigorously,⁴⁶⁾ but long-term data may be more challenging to interpret; and (4) the chronic immobilized stress model used in these studies could not completely mimic human CPS. Thus, it is too hard to fully explore the mechanism of CPS-related CVD and these drug-mediated cardiovascular benefits using these animal models.

Disclosure

Conflicts of interest: The authors declare that they have no conflicts of interest to disclose with respect to this manuscript.

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