

Review article

DPP-4/GLP-1 axis as a potential therapeutic target in chronic stress-related vascular aging and atherosclerosis

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Running title: Wan et al. GLP-1/DPP-4 axis in atherosclerosis

Word count: 4905, Figures: 1, Tables: 1

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Abstract

Exposure to psychosocial stress is a risk factor for cardiovascular disease, including vascular aging, angiogenesis, and atherosclerosis-based cardiovascular disease (ACVD). Dipeptidyl peptidase-4 (DPP-4) is a complex enzyme (also called CD26) that acts as a membrane-anchored cell surface exopeptidase. DPP4 is upregulated in metabolic and inflammatory cardiovascular disorders. The widespread expression of DPP4 in macrophages and immune cells and the noncatalytic function of DPP4 (also called CD26) as a signaling and binding protein across a wide range of species suggest a teleological role for DPP4 in inflammation and immune response. DPP-4 exhibits many physiological and pharmacological functions by regulating its extremely abundant substrates [e.g., stromal cell-derived factor-1 α / C-X-C chemokine receptor type-4, glucagon-like peptide-1 (GLP-1), etc.]. Over last ten year, emerging data demonstrated unexpected roles for GLP-1 and DPP-4 in extracellular and intracellular signaling, immune activation, inflammation, oxidative stress production, cell apoptosis, insulin resistance, and lipid metabolism,. This mini review has focuses on recent novel findings in this field, highlighting an imbalance between GLP-1 and DPP4 as a potential therapeutic molecular target in treatments of chronic psychological stress-related atherosclerotic cardiovascular disease in humans and animals.

Key words: chronic stress, vascular senescence, atherosclerosis, inflammation, dipeptidyl peptidase-4,

Chronic psychological stress (CPS) is considered a risk factor for vascular aging and atherosclerosis-based cardiovascular disease (ACVD) based on clinical and experimental (Table 1).¹⁻⁵ 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 past several decades.⁶⁻¹¹ 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).¹² 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 (CABG) and AMI is known, and it is well documented that transient psychological stresses may cause potentially fatal arrhythmias and acute cardiovascular events.¹³⁻¹⁸ Last ten years, it has been established that the chronic psychological stressors in modern lifestyles are closely associated with the incidence of hypertension, metabolic syndrome, diabetes mellitus (DM), and CVD.¹⁹⁻²⁴ Clinical and laboratory findings from our team and other groups showed that chronic psychological stressor activates intracellular and extracellular pathways (including the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system) by eliciting pathophysiological over-actions, resulting in metabolic and inflammatory cardiovascular disorders (Table 1).²⁵⁻³⁰ The precise mechanisms involved in stress-related vascular aging and atherosclerotic lesion formation and progression remain largely uncertain, however.

Dipeptidyl peptidase-4 (DPP-4) is a complex enzyme that acts as a membrane-anchored cell surface exopeptidase that transmits intracellular signals

through a small intracellular tail.³¹⁻³³ 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 a useful antidiabetic drugs was based on the concept that these agents would enhance systemic and tissue glucagon-like peptide-1 (GLP-1) levels, causing an improvement of the insulinotropic effects of blood sugar.³⁴⁻³⁷ In addition to GLP-1-dependent effects on the cardiometabolic risk profile, DPP-4 inhibition represents vascular protective beneficial effect via the modulation of several substrate factor activities (e.g., colony-stimulating factor, stromal cell-derived factor-1 α , granulocyte-colony-stimulating factor, granulocyte macrophage-colony-stimulating factor, neuropeptide Y, and high-mobility group box 1).³⁸ A recent study reported that individuals with and without diabetes mellitus had increased plasma DPP-4 levels and decreased plasma GLP-1 levels.³⁹ In mice and rats, chronic stress increased circulating and tissue DPP-4 activities and decreased plasma and brain GLP-1 levels,⁴⁰⁻⁴² 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.

Although a growing body of evidence indicates that DPP-4 plays an important role in the initiation and progression of ACVD,⁴³⁻⁴⁵ 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 DPP4 levels.^{26, 42} It is well-known that inflammation participates in all stages of atherosclerosis, including initiation, progression, calcification, plaque rupture, and ultimately, the thrombotic complications.^{46, 47} Data from our research 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 1), leading to an increased output of inflammatory monocytes and neutrophils.^{25, 26} Existing evidence has confirmed that stress can increase inflammatory actions in vascular and adipose tissues.^{7, 41} 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 significantly rectified by DPP-4 inhibitor anagliptin treatment (Fig. 1).⁴⁸ Accumulating evidence suggests that oxidative stress also plays a critical role in vascular senescence and atherosclerotic plaques in animals and humans.⁴⁹ The observations described herein exhibit that anagliptin mitigated NADPH oxidase component expression (p22phox, p47phox, p67phox, and gp91phox) and superoxide (O_2^-) generation. Moreover, the levels of adiponectin (APN) protein and gene were increased in blood, inguinal and subcutaneous adipose tissues of stressed apoE lipoprotein-deficient (ApoE)^{-/-} mice, and these changes were reversed by DPP4 inhibition.⁴⁸ In vitro, the GLP-1 analogue exenatide increased APN expression in adipose tissue-derived immature adipocytes in a dose-dependent manner, whereas anagliptin did not affect on it.⁴⁸ Surprisingly, APN depletion with its neutralizing antibody almost completely diminished the anagliptin-mediated vascular benefits in ApoE^{-/-} mice loaded a high-fat diet.⁴⁸ APN can protect against various vascular injuries under conditions of stress.⁵⁰ 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 APN-induced anti-oxidative stress production and

anti-inflammation in ApoE^{-/-} mice under our experimental conditions (Fig. 1). This notion was further supported by the findings of a comparable effect of exenatide on stress-related vascular harmful changes in ApoE^{-/-} mice fed high fat-diet.⁴²

Accumulating investigations 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 (Fig. 1), and that these inflammatory cytokines increase the degradation of extracellular matrix proteins (collagen and elastin).⁵¹⁻⁵⁴ Novel insights into those proteases have been performed possible by the generation and in-depth analyses of transgenic and knockout mice.⁵⁵⁻⁵⁷ 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, and cell events (apoptosis, transmigration and proliferation), neovascularization and matrix protein metabolism.⁵⁸⁻⁶⁰ Pharmacological inhibitions targeting GLP-1 receptor stimulation and DPP-4 activity exhibited a protective effect on the expressions and/or activities of proteolytic enzymes [e.g., cathepsin L (CatL), CatS, CatK, MMP-9, and MMP-9] and matrix protein metabolism (elastin and collagen) in the lesions of stressed animals fed a high-fat diet.^{41, 42, 48} These therapies also suppressed the levels of plaque peroxisome proliferator-activated receptor- α (PPAR- α) and angiotensin II type 1 receptor (AT1R) proteins.⁴⁸ Both receptor systems with their ligands have been exhibited to regulate CatS/K and MMP-2/-9 expressions via the enhancement of the productions of oxidative stress and inflammatory cytokines in both of vivo and in vitro.^{59, 61} In vitro, exenatide suppressed tumor-necrosis factor-alpha (TNF- α)

expression in cultured macrophages. These findings thus suggest that atherosclerotic lesion development with neovascularization and instability may be attributable to the increase of MMP-2/-9- and CatL/S/K-mediated proteolysis induced by the stimulation of PPAR- α - and AT1R-singling pathways-related oxidative stress production and inflammation in animals under chronic stress conditions (Fig. 1).

Previous clinical and basic researches reported that GLP-1 and DPP-4 activity involved in lipid metabolism has been indicated.^{42, 43, 48} Biological analyses demonstrated that anagliptin reduced blood non-esterified fatty acid and triglyceride in stressed animals,⁴⁸ and similar results were found in stressed animals treated by exenatide.⁴² Exenatide dramatically reduced the foam cell formation of peripheral blood monocyte-derived macrophages.⁴² Clinical observations have provided a limited beneficial effect of DPP-4 inhibition on plasma apolipoprotein B-48 and triglyceride levels; for a review see.⁴³ Therefore, the improvements of free fatty acid and triglyceride metabolism may also contribute to the incretin-based glucose-lowering drug-related vascular benefits in mice under stress (Fig. 1). However, it should be figured out that these treatments had no an inhibitor effects on the plasma levels of 'good' and 'bad' cholesterols (i.e., high- and low-density lipoprotein cholesterols) in animals under our experimental stress conditions.^{46, 52} Further clinical researches are needed to study this issue.

Recently, it was reported that increased circulating DPP-4 and decreased circulating APN and GLP-1 might be as new useful biomarkers to predict the presence of stress in animals.^{26, 40} 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 for the assessment of brain

injury in animals that have been subjected to chronic stress.²⁶ 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 in a large scale prospective or/and retrospective cohort studies.

Overall, the recent findings from the Lei and colleagues study suggest a potential clinical applicable benefit of DPP-4 inhibitors on vascular senescence and atherogenesis in ApoE^{-/-} mice under experimental stress conditions,⁴⁸ with an effect size similar to that of GLP-1 analogue-based therapies.⁴² This protective effect is supported by the finding of a consistent effect of genetic inhibition targeting DPP-4 on inflammation cell production and output into the peripheral blood of rats under chronic stress.²⁶ These results are consistent with the positive findings of a small clinical trial of GLP-1 analogue or DPP-4 inhibitor as a useful treatment to mitigate atherosclerotic lesion formation and coronary artery events in ACVD patients with and without DM.^{37, 62} Given the costs and safety profile of DPP-4 inhibitors, these intriguing data, notwithstanding some limitations (e.g., lacking significant dose-dependent actions [30 mg/kg/d, 60 mg/kg/d 120 mg/kg/d] etc.), make a compelling sense for the further study of the effects of GLP-1 analogues and DPP-4 inhibitors in CPS-related ACVD. However, it should be noted that the use of DPP4 inhibitors in individuals with DM (unknown with or without psychological stressors) is not fully supported until its beneficial effects can be revealed in large-scale long-term clinical trials (e.g., a saxagliptin trial: cardiovascular events, especially the rate of hospitalization for heart failure).³⁴ Further research (including sub-analyses) is necessary to study the clinical uses of DPP4 inhibitors.

Authors contributions

Writing-Original draft: Wan Y: Writing-review and editing: Piao L and Cheng XW.

Sources of Funding

This work was supported in part by the Scientific Research Fund of the Chinese Ministry of Education (nos. 81560240, 81460082, 81660240, and 81770485).

Acknowledgements

No conflict of interest/

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Table 1. Experimental studies of chronic stressors on vascular aging, angiogenesis and atherosclerosis

Diseases & implications	Animals Publication year/journal	Stressor	Treatment	Mechanism	Morphological and functional alterations	Ref
Angiogenesis	BALB/c (2015) Atherosclerosis	Immobilized stress (3-weeks)	Fluoxetine hydrochloride (18 mg/kg/d)	Oxidative stress↓/ VEGF↑/ p-Erk1/2↑	Blood flow↑ Capillary density↑	5
Metabolic disorder Prothrombosis	C57BL/6j (2012) Diabetes	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-Apoet m1Unc/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} CD 150 ^{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/d) Exenatide (5μg/kg/d) A specific Adrβ3 inhibitor (L748337: 0.5mg/kg/d)	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} CD 150 ^{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/d)	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) Psychoneuroendo crinology	Immobilized stress (2-weeks)	DPP4 inhibitor (alogliptin: 15 or 45 mg/kg/d)	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	C57BL/6j	Immobilized	OX inhibitor	Plasma and tissue	Macrophage	30

dysorder Hyperuricemia	(2017) Scientific Resports	stress (2-weeks)	(febuxostat: 1 or 5 mg/kg/d)	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↑	infiltration↓ Hyperuricemia↓ Glucose Dysmetabolism↓ Prothrombotic state↓	
Vascular aging Atherosclerosis	ApoE ^{-/-} mice (KOR/Stm Slc-ApoE ^{shl} , BALB/c background) (2017) International Journal of Cardiology	Chronic variable stress (12-weeks)	DPP4 inhibitor (anagliptin: 30mg/kg/d) APN neutralizing antibody (450 µg/kg/d)	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↓ SMCs contents(-)	31
Vascular aging Angiogenesis neovascularizat ion	C57BL/6j DPP4 ^{-/-} rats APN ^{-/-} mice (2017) Journal of American Heart Association	Chronic variable stress (4-weeks)	Anagliptin (low: 30mg/kg/d; high: 60mg/kg/d) Exenatide (5µg/kg/d) APN neutralizing antibody (450mg/kg/d)	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-Biologi cal Interactions	Chronic immobilized stress (2-weeks)	Anagliptin (30mg/kg/d)	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 ^{shl} , BALB/c background) (2017) Atherosclerosis	Chronic variable stress (12-weeks)	GLP-1 analogue (exenatide: 5µg/kg/d)	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↓ SMCs contents(-)	42

Abbreviations: 7ND, dominant negative mutation of monocyte chemoattractant protein-1;
MCP-1, monocyte chemoattractant protien-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 production; 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-AMPK α , phospho-AMP-activated protein kinase α ; PPAR- γ ; peroxisome proliferator activated factor- γ ; PGC-1 α , PPAR- γ co-activator; eNOS, endothelial nitric oxide synthase; DPP4^{-/-}, DPP4 deficiency; ApoE^{-/-}, apolipoprotein deficiency; APN^{-/-}, adiponectin deficiency; Ref., reference; (-) indicates no change; \uparrow indicates increasing; \downarrow indicates decrease or improvements.

Figure 1. The proposed mechanisms of how GLP-1R activation and DPP4 inhibition suppress stress-related vascular endothelial senescence and atherosclerotic lesion formation in mice fed a high-fat diet. Stress enhanced the levels of blood DPP4 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, chronic psychological stress; DPP4, dipeptidyl peptidase-4; APN, adiponectin; PPAR- γ , peroxisome proliferator activator factor- γ ; Adr β 3, β 3 adrenaline receptor; GLP-1R, GLP-1 receptor; PGC-1 α , PPAR- γ co-activator-1 α ; GLP-1, glucagon-like peptide-1; SMC, smooth muscle cells; ECs, endothelial cells; Mac, macrophages; HSC, hematopoietic stem cell; MMP-2, matrix metalloproteinase-2; CatS, cathepsin S.

Figure 1

