Pleiotropic effects of the dipeptidylpeptidase-4 inhibitors on the cardiovascular system

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1Department of Internal Medicine, University of Missouri School of Medicine, Columbia, Missouri; 2Department of Medical Pharmacology and Physiology, University of Missouri School of Medicine, Columbia, Missouri; 3Diabetes and Cardiovascular Center, University of Missouri, Columbia, Missouri; and 4Harry S. Truman Veterans Affairs Medical Center, Columbia, Missouri

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Aroor AR, Sowers JR, Jia G, DeMarco VG. Pleiotropic effects of the dipeptidylpeptidase-4 inhibitors on the cardiovascular system. Am J Physiol Heart Circ Physiol 307: H477–H492, 2014. First published June 14, 2014; doi:10.1152/ajpheart.00209.2014.—Dipeptidylpeptidase-4 (DPP-4) is a ubiquitously expressed transmembrane protein that removes NH₂-terminal dipeptides from various substrate hormones, chemokines, neuropeptides, and growth factors. Two known substrates of DPP-4 include the incretin hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide, which are secreted by enteroendocrine cells in response to postprandial hyperglycemia and account for 60–70% of postprandial insulin secretion. DPP-4 inhibitors (DPP-4i) block degradation of GLP-1 and gastric inhibitory peptide, extend their insulinitropic effect, and improve glycemia. Since 2006, several DPP-4i have become available for treatment of type 2 diabetes mellitus. Clinical trials confirm that DPP-4i raises GLP-1 levels in plasma and improves glycemia with very low risk for hypoglycemia and other side effects. Recent studies also suggest that DPP-4i confers cardiovascular and kidney protection, beyond glycemic control, which may reduce the risk for further development of the multiple comorbidities associated with obesity/type 2 diabetes mellitus, including hypertension and cardiovascular disease (CVD) and kidney disease. The notion that DPP-4i may improve CVD outcomes by mechanisms beyond glycemic control is due to both GLP-1-dependent and GLP-1-independent effects. The CVD protective effects by DPP-4i result from multiple factors including insulin resistance, oxidative stress, dyslipidemia, adipose tissue dysfunction, dysfunctional immunity, and antiapoptotic properties of these agents in the heart and vasculature. This review focuses on cellular and molecular mechanisms mediating the CVD protective effects of DPP-4i beyond favorable effects on glycemic control.

vascular dysfunction; obesity; insulin resistance; diastolic dysfunction; incretin

Epidemiological studies reveal that two out of three American adults are overweight or obese and at increased risk for progression to type 2 diabetes mellitus (T2DM) (58). Consumption of a Western-style diet, high in fat, high-fructose corn syrup, and salt, in concert with a sedentary lifestyle are major factors contributing to the pandemics of obesity and T2DM (96, 128). Among overweight/obese individuals there is a high incidence of insulin resistance that is a seminal risk factor for progression to cardiac, renal, and vascular maladaptive structural and functional abnormalities in individuals who are prediabetic, as well as those with T2DM (176). T2DM is classically recognized as a metabolic disorder yet develops as a collection of metabolic and immune derangements that predictably increase the risk for cardiovascular (CV) disease (CVD) and chronic kidney disease (CKD). The high incidence of CVD in T2DM in the setting of obesity can be explained, in part, by the interaction of several systemic maladaptations, such as, insulin resistance, chronic activation of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system with associated increases in inflammation, oxidative stress, and maladaptive immune responses (175). The vasculature is exquisitely sensitive to all of these abnormalities; consequently, vascular dysfunction is often the first derangement to occur in the progression to end organ/tissue damage in T2DM.

Although the conventional goal of diabetes therapies is to reduce plasma glucose and glucose toxicity (glycemic control), it has become increasingly apparent that development of novel safe and effective therapeutic strategies to improve long-term glycemic control should also have favorable direct and/or indirect effects on CVD and CKD outcomes. This more contemporary therapeutic approach is due, in part, to recent observations that some conventional diabetes therapies, although effective at controlling glycemia, may actually increase the risk of CVD events, increase hypoglycemic episodes and result in...
weight gain. As such, in 2008, the Food and Drug Administration issued more stringent testing requirements for drug companies to ensure that new diabetes drugs do not increase CVD risk (59). Indeed, it is known that the incidence of CVD events associated with T2DM can be reduced by therapeutic interventions targeting multiple CV and metabolic derangements inherent with T2DM, rather than interventions targeting a single risk factor, such as hyperglycemia (62). Therefore, there is a need for drugs that are effective not only for controlling glycaemia, but also for improving CVD outcomes independently of glycaemic control.

Accumulating evidence suggests that the modulation of incretin signaling by glucagon-like peptide-1 (GLP-1) agonists is beneficial in both glycemic control, as well as CV protection (182). In this regard, dipeptidylpeptidase-4 (DPP-4) inhibitors (DPP-4i), which have been developed specifically as incretin-based oral antihyperglycemia therapies, are now emerging as novel agents that have the potential to reduce the progression to CVD and CKD. DPP-4i exhibit multiple protective effects that collectively contribute to improvement in vascular function which can reduce the risk for development of vascular disease, heart failure (HF), and CKD (Fig. 1). The beneficial effects of these drugs, beyond glycemic control, occur by both GLP-1-dependent and -independent mechanisms (147, 150).

Although most preclinical and clinical studies support a positive role for DPP-4i in the vasculature, a few studies indicate a negative impact on vascular function (11, 147, 167, 192). For instance, an increase in the rate of hospitalization has been reported for HF in patients treated with saxagliptin (3.5 vs. 2.8% by 2-yr Kaplan-Meier analysis), despite improvements in glycemic control (167). In another report, attenuation of flow-mediated dilation by sitagliptin and alogliptin has been reported (11). In contrast, vildagliptin improved endothelial function and alogliptin attenuated postprandial decrease in flow-mediated dilation in subjects without diabetes (142, 183). Further large-scale CV outcome studies will resolve the issue of excess HF risk. Importantly, the safety and beneficial effects of DPP-4i continue to be evaluated in several large CV outcomes studies, including the Trial to Evaluate Cardiovascular Outcomes after Treatment with Sitagliptin (TECOS) (69), Cardiovascular Outcomes Study of Alogliptin in Subjects With Type 2 Diabetes and Acute Coronary Syndrome (EXAMINE) (190, 191), Saxagliptin and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus (SAVOR-TIMI) (167), and Cardiovascular Outcome Study of Linagliptin Versus Glimepiride in Patients With Type 2 Diabetes (CAROLINA) (160). Although the use of GLP-1 receptor (GLP-1R) agonists and DPP-4i have been shown to be associated with develop-
ment of pancreatitis and pancreatic and thyroid cancer in a few studies (26, 49), such adverse outcomes have not been demonstrated in many recent preclinical and clinical studies (46, 64, 111, 140, 167, 190).

DPP-4 Biology and Pharmacology

The incretin hormones GLP-1 and gastric inhibitory peptide (GIP) are secreted by enteroendocrine cells in response to postprandial hyperglycemia and account for 60–70% of postprandial insulin secretion (the incretin effect). Early studies demonstrated that the incretin effect was impaired in T2DM (139, 141). The recognition of the fundamental glucoregulatory role played by the intestinal-derived incretin hormones GLP-1 and GIP eventually led to the recent development of novel targeted therapies for treatment of T2DM. GLP-1 and GIP are key in regulating both postprandial and long-term glucose homeostasis by augmenting glucose-dependent pancreatic insulin secretion, suppressing glucagon release, slowing gastric emptying, enhancing satiety, and modulating the so-called “gut-brain axis” (24, 47). Once secreted, incretin hormones have a half-life of 1 to 2 min in plasma because of rapid degradation by the ubiquitous enzyme DPP-4. As such, direct administration of GLP-1 for lowering glucose levels may be limited because of enzymatic action of circulating DPP-4, which tends to be elevated especially in the setting of obesity (9, 100, 101, 107). One strategy to address this limitation has been the development of drugs to specifically inhibit DPP-4 to reduce catabolism of active GLP-1 and enhance its bioavailability. Several DPP-4i, including sitagliptin, saxagliptin, vildagliptin, linagliptin, and alogliptin, are now approved for use in the United States. Clinical trials have demonstrated that DPP-4i, alone or in combination with metformin or sulphonylureas, lower hemoglobin A1c (HbA1c) levels 0.6–0.9%, have low incidence of hypoglycemia, and are well tolerated because of a low incidence of side effects (90, 93, 133, 185).

The clinical pharmacology of DPP-4i inhibitors has been the subject of several recent comprehensive reviews and will not be discussed in detail here (12, 67, 163). Although DPP-4 inhibitors are structurally diverse, in general, they possess similar pharmacodynamic properties. As a monotherapy, or as an adjunct to conventional glucose lowering agents, DPP-4i are comparatively effective at elevating plasma GLP-1 levels and lowering blood glucose and HbA1c, are generally weight neutral, and have similar safety and tolerability profiles (1, 3, 83, 134, 180). On the other hand, the pharmacokinetic profiles of the various DPP-4i compounds indicate differences in half-life, metabolism, distribution and bioavailability, plasma protein binding, and route of elimination that could have clinical relevance. Impaired kidney function, a common comorbidity in obesity and diabetes, is known to alter the elimination of alogliptin, sitagliptin, saxagliptin, and vildagliptin (67). As such, dose reduction is generally indicated in relation to the severity of renal dysfunction. In this regard, it is noteworthy that linagliptin is eliminated largely unmetabolized by a biliary/hepatic route, rather than by renal elimination like other DPP-4i. As such, linagliptin can be prescribed to patients with renal insufficiency without the need to decrease the dose (67).

Most studies demonstrating vasculoprotection or cardioprotection by GLP-1 have used supraphysiological concentrations of native GLP-1 or GLP-1 analogs that elicit GLP-1 signaling beyond what is physiological. DPP-4i have been reported to induce a modest increase in circulating active GLP-1 levels above baseline (~2- to 6-fold) in diabetic mice, rats, and humans (9, 40, 144, 155). Thus the extent of GLP-1-dependent responses to DPP-4i in the vasculature may be somewhat limited relative to those evoked in experimental studies by GLP-1 analogs. Moreover, DPP-4i may have the potential to exert either a broader range of beneficial effects or a different subset of benefits on overall CVD and CKD outcomes compared with cardioprotective and vasodilatory effects of GLP-1 analogs (15, 182). Accumulating evidence suggests that DPP-4i possess anti-inflammatory, antioxidant, and antipoptotic effects in the vasculature and that these beneficial effects may also occur independent of GLP-1 (Fig. 2). This notion is based on the fact that DPP-4, in addition to its actions on incretin hormones, also acts on a number of other substrates, e.g., stromal cell-derived factor 1α (SDF-1α), as well as binds to specific proteins (e.g., fibronectin) that can contribute to the pathophysiology of CVD (202). Therefore, the GLP-1-independent effects of DPP-4i may include suppression of harmful effects of DPP-4. In this regard, administration of stable GLP-1 analogs does not affect DPP-4 activity that is often increased in plasma from patients with obesity, diabetes, or HF (107, 156). GLP-1 analogs are reported to result in small blood pressure (BP) reductions and modest weight loss (182). Some, but not all, studies have demonstrated small reductions in BP with DPP-4i (9, 114, 132, 145, 148, 152), and these effects may be independent of effects associated with weight loss, as DPP-4i tend to be body weight neutral. Given the advantage of oral administration of DPP-4i and their CV safety profile, these drugs are becoming second- and third-line therapies for glycemic reduction in diabetic patients. Importantly, emerging evidence suggests that DPP-4i may have neutral (167, 190) or positive effects on CVD outcomes (4, 89, 91, 182).

DPP-4 is a 766-amino-acid membrane protein (110 kDa) that was originally designated as lymphocyte cell surface protein, cluster of differentiation-26/ antigen (CD26) for its role in T-cell activation. However, it is also expressed in other immune cells and nonimmune cells/tissues (5, 202). Tissues/organs expressing DPP-4 include blood vessels, adipose tissue, kidney, liver, pancreas, lymph nodes, spleen, bone, brain, lung, prostate, thymus, and uterus (109, 202). Various cytokines, such as interleukin (IL)-12 and interferon-γ, as well as transcription factors, such as hepatocyte nuclear factor-1α and nuclear factor-κB, regulate CD26/DPP-4 expression in various cells (16, 50). CD26/DPP-4 expression on CD4+ and CD8+ T cells has been reported to be higher in T2DM patients and reduced with active glucose control (109).

In addition to membrane-associated DPP-4 (mDPP-4), there is a soluble circulating form of DPP-4 (sDPP-4) that lacks the transmembrane and cytoplasmic domains of mDPP-4 and is largely responsible for degradation of the majority of newly synthesized GLP-1 and GIP (42, 73). Evidence suggests that proteolytic cleavage of mDPP-4 is the major source of circulating sDPP-4 (107). Although liver epithelium and lymphocytes are often cited as sources of sDPP-4 (37), the sources of DPP-4 and factors that regulate circulating levels are largely unknown. In this regard, a recent study demonstrated that adipose tissue is a significant source of sDPP-4 and that insulin and tumor necrosis factor-α can induce shedding of sDPP-4 (107). Thus the elevated levels of sDPP-4 reported in obesity/
T2DM may be related to the consequences of insulin resistance (e.g., elevated insulin and/or glucose), hyperglycemia, and adipose tissue inflammation (elevated tumor necrosis factor-α), which promote DPP-4 shedding from adipose tissue (107). Insulin resistance is associated with increased expression and release of DPP-4 (107, 202).

DPP-4 specifically cleaves dipeptides from incretin as well as a number of non-incretin peptide substrates containing a penultimate proline or alanine residue at the NH2-terminus (131). Importantly, some of these peptides, including stromal-cell derived factor-1 (SDF-1α), brain natriuretic peptide (BNP), neuropeptide Y (NPY), and peptide YY (PYY), have direct or indirect effects in the vasculature (Fig. 2). The effects of these substrates add considerable complexity to the potential mechanisms by which DPP-4i mediate effects on vascular/endothelial function. However, most of these peptides mediate a wide range of beneficial pleiotropic effects in the vasculature that are not imparted by GLP-1 agonists alone, although some DPP-4 substrates, such as NPY, exert vasoconstrictor effects in the setting of hypertension (182). Since DPP-4 activity is increased in obesity and T2DM, it is possible that inhibition of DPP-4 will not only increase GLP-1 but also enhance the activities of several beneficial substrates.

In addition to enzymatic actions, DPP-4 also has additional nonenzymatic functions involving binding to adjacent membrane proteins, including adenosine deaminase, caveolin, kidney, Na⁺/H⁺ exchanger, thromboxane A2 receptor, and C-X-C chemokine receptor type 4, as well as binding to extracellular matrix proteins such as fibronectin and collagens (202) (Fig. 2). For instance, the cysteine-rich region of DPP-4 contains binding sites for collagen and fibronectin that enable DPP-4 containing inflammatory T-helper cells to concentrate in areas of accumulating extracellular matrix proteins, such as those found in and around an affected blood vessel undergoing fibrosis and associated vascular stiffness (Fig. 3).

**DPP-4i and Vasculoprotection**

Emerging evidence suggests that DPP-4i may have beneficial pleiotropic effects in the vasculature and heart that may reduce the risk of CVD, including hypertension, atherosclerosis, stroke, and heart, and CKD (Fig. 1).

**Hypertension.** Approximately 70% of patients with diabetes have high BP, meaning they are twice as likely to have hypertension compared with individuals without diabetes (106). BP responses to DPP-4i therapy have been reported to be either neutral with saxagliptin and linagliptin (35, 185) or modestly reduced with sitagliptin (13, 105, 132, 133, 145). Most relevant clinical trials were of short and medium term; results of long-term trials are not yet available, so it cannot be concluded definitively that DPP-4i therapy is BP neutral in hypertensive diabetic humans.

In rodent models, sitagliptin and saxagliptin reduced BP in spontaneously hypertensive rats (SHRs) and young prehypertensive SHRs (114, 122, 148). Sitagliptin also reduced BP in Zucker diabetic fatty rats (56). We recently reported that linagliptin reduced radiotelemetric-derived measures of mean arterial pressure in mildly hypertensive, insulin-resistant Zucker obese (ZO) rats (9). The elevated BP in untreated ZO rats was associated with impaired endothelium-dependent vasodilation of small arterioles, and this endothelial defect was normalized by treatment with linagliptin.
In addition to reducing the enzymatic degradation of GLP-1 and GIP, DPP-4i decrease the degradation of several vasoactive peptides, such as BNP, substance P, PYY receptor (1–36), and NPY (22). Thus there is potential for DPP-4i therapy to either raise or lower BP based on these multiple actions and differing effects have been reported. To this point, DPP-4i therapy may be contraindicated in hypertensive patients with T2DM taking angiotensin-converting enzyme (ACE) inhibitors (ACEi) to lower BP. A recent study showed that sitagliptin increased BP in subjects with the metabolic syndrome (121). In this study, sitagliptin monotherapy slightly decreased BP and augmented the hypotensive effects of a submaximal dose of enalapril (5 mg/day). Interestingly, the combination of sitagliptin and a higher dose (10 mg/day) of enalapril that maximally inhibits ACE, neutralized the BP lowering effect of enalapril, and increased heart rate and circulating norepinephrine levels, suggesting that sitagliptin induces sympathetic nervous system activation under conditions of maximal ACEi. It has been suggested that the mild antihypertensive effect of DPP-4i in the absence of ACEi or in combination with a low dose of ACEi is mediated by attenuation of sympathetic nervous system activation by substance P and decreased degradation of NPY (1–36), inactivated substance P, and increased sympathetic outflow to attenuate the antihypertensive effect of ACEi (86). These results were consistent with those from a study in SHRs showing that a combination of sitagliptin and ACEi increases BP (87). Taken together, these studies suggest that during combination DPP-4i and high-dose ACEi, activation of the sympathetic nervous system by substance P and decreased...
Alzheimer’s disease GLP-1 analogs have been shown to amend nondiabetic rodent models (21, 39, 108). In rodent models of nist, exendin-4, reduces the severity of stroke in diabetic and experimental animal studies demonstrate that the GLP-1R agonist incretin enhancer therapy could be neuroprotective (79). Ex-cerebral blood brain barrier (82), suggesting the possibility that hypertension, renal function, and glycemic control may be more effectively managed in patients with T2DM treated with a combination of ARB/DPP-4i as opposed to ACEi/DPP-4i.

Atherosclerosis. DDP-4i therapy may reduce the risk for further development of atherosclerosis via improved glucose regulation; however, there may be additional DPP-4i-mediated molecular mechanisms involved in blunting atherosclerosis or stabilizing plaque formation. In the setting of obesity/T2DM, a combination of hyperglycemia, elevated levels of circulating triglyceride (TG)-rich lipoproteins, and proinflammatory cytokines secreted from inflamed adipose tissue activates the endothelium, resulting in impaired insulin signaling, reduced nitric oxide (NO) synthesis and signaling, and recruitment of inflammatory cells to the vessel wall to incite conditions leading to atherosclerosis. The endothelium is the first barrier breached in the progression of atherosclerosis and damage and remodeling stem from a coordinated lipid and inflammatory cell infiltration (117). The beneficial effects of DPP-4i, independent of glycemic control, may occur through their effects on GLP-1 or GLP-1 split peptides. In this regard, a recent study demonstrates that GLP-1(7–37) and its metabolites, GLP-1(9–37) and GLP-1(28–37) reduce inflammation within atherosclerotic plaques in the aorta of apolipoprotein E (ApoE)−/− mice and improve plaque stability by decreasing the degradative actions of matrix metalloproteinase (MMP)-9 in the fibrous cap (25). Moreover, these split products also decreased plaque macrophage content, as well as expression of MMP-9. These anti-inflammatory effects were similar to GLP-1(7–37). Despite similar efficacy these compounds may work through different pathways to achieve plaque stabilization. Evidence suggests that in the absence of GLP-1R, GLP-1(9–37) and GLP-1(28–37) exert protective effects in the cardiovascular system (14, 15). Like GLP-1(7–37), GLP-1(9–37) does increase adenosine 3′,5′-cyclic monophosphate (cAMP) concentrations suggesting the possibility of similar signal transduction by an unidentified G protein-coupled receptor. Furthermore, the potential anti-atherogenic properties of DPP-4i may arise from GLP-1 independent mechanisms through other DPP-4 substrates or pleiotropic effects of DPP-4i as discussed in section 4 below.

Stroke. T2DM is major risk factor for degenerative neurological diseases, such as stroke (99) and Alzheimer’s disease (158). GLP-1Rs are expressed in neurons from rodents and humans (66, 72), and GLP-1 and GLP-1 analogs readily cross the blood brain barrier (82), suggesting the possibility that incretin enhancer therapy could be neuroprotective (79). Experimental animal studies demonstrate that the GLP-1R agonist, exendin-4, reduces the severity of stroke in diabetic and nondiabetic rodent models (21, 39, 108). In rodent models of Alzheimer’s disease GLP-1 analogs have been shown to ameliorate disease progression (130). Unlike GLP-1 or GLP-1 agonists, DPP-4i have not been reported to cross the blood brain barrier, and this raises the question whether DPP-4i exhibit comparable neuroprotection like that shown for GLP-1 analogs. A recent clinically relevant study tested the antistroke efficacy of linagliptin and a comparator glimepiride in middle-aged nondiabetic and diabetic mice (40). Stroke was induced by middle cerebral artery occlusion and T2DM was induced by high-fat diet feeding. Glimepiride was used because it lowers blood glucose but has no effect on incretin hormones. Linagliptin treatment reduced plasma DPP-4 activity and increased GLP-1 levels. This was associated with a significant increase in the number of surviving cortical neurons, despite no reduction in infarct size, in both nondiabetic and obese diabetic mice. Linagliptin reduced hyperglycemia in the diabetic model but did not affect glycemia in the nondiabetic model, suggesting that the efficacy of linagliptin in reducing stroke does not depend on glucose reduction. Like linagliptin, glimepiride was efficacious in reducing severity of stroke in nondiabetic mice but had little efficacy in the diabetes model. In a randomized, double-blind, noninferiority trial testing the 2-yr safety and efficacy of linagliptin versus glimepiride in patients with T2DM receiving metformin, but having inadequately controlled HbA1c levels, there were significantly fewer major CVD events (12 vs. 26% for linagliptin vs. glimepiride, respectively) (63). These findings were mostly attributed to a lower incidence of nonfatal stroke events in patients treated with linagliptin compared with glimepiride (P = 0.032). Although adjunctive linagliptin and glimepiride therapies were equally efficacious at improving glycemia, linagliptin was less likely to lead to hypoglycemic events or weight gain that was associated with glimepiride. The possible mechanisms for the observed antistroke efficacy of linagliptin are unknown at this point, but it is likely to be due, at least in part, to GLP-1-mediated effects in the brain as was indicated in a similar study using exendin-4 (39). In this regard, modulation of MMPs have been implicated in the pathogenesis of stroke (31, 92), and GLP-1 agonists suppress MMP-9 activity (143).

Heart disease. A recent clinical study reported that diastolic, but not systolic, dysfunction is a highly prevalent (40%) comorbid condition in a large population of patients with early phase T2DM and no history of CVD (151). In the setting of overnutrition/obesity, diastolic dysfunction is often the earliest functional cardiac abnormality (161, 184, 199). Moreover, prediabetic insulin resistance and diastolic dysfunction may become more prevalent given the emerging pandemic in childhood/adolescent overweight/obesity (146). We recently tested the notion that the DPP-4i, linagliptin, could be useful in ameliorating pathophysiologic abnormalities in diastolic and vascular endothelial dysfunction in a clinically relevant rodent model of obesity associated with insulin resistance. We treated ZO rats with linagliptin for 8 wk (9), beginning at 8 wk of age when they exhibit both insulin resistance and diastolic dysfunction (203), CV manifestations that are seen in young obese humans with cardioenal metabolic syndrome (176). Linagliptin markedly improved impaired diastolic function, and this was associated with improved vascular endothelial function and a reduction in BP.

DPP-4i could also be effective at improving cardiac function in more severe forms of heart disease associated with myocardial infarction (182, 200). A recent report demonstrated a
correlation between circulating DPP-4 activity and cardiac dysfunction in patients with HF and in a rodent model of experimental HF (45). Earlier studies demonstrated activation of the cardioprotective signaling pathways by GLP-1, leading to improvement in coronary blood flow (172, 201), decreases in cardiomyocyte apoptosis (157), and reduction in infarct size following ischemia-reperfusion (I/R) injury (18, 143). In the setting of severe diastolic dysfunction, left atrial dilation, and abnormal myocardial perfusion, it has been shown that the foremost contributor to advanced left ventricular (LV) diastolic dysfunction was an ischemic myocardium (151). Thus the increased bioavailability of GLP-1 consistently observed with DPP-4i therapy could confer cardioprotection, especially in the ischemic myocardium. DPP-4i have been tested in experimental rodent models of myocardial infarction and ischemia and shown to have mostly positive effects (36, 76, 78, 162, 197, 198). In one study, linagliptin significantly reduced infarct size and area of fibrosis in male Wistar rats after I/R injury both in the short term (7 days post I/R) and long term (8 wk post-I/R) (78). Cardiac function was impaired in this model following I/R injury, yet diastolic function, as measured by a significant improvement in the maximum rate of LV pressure decline, was improved 8 wk following the I/R procedure. Linagliptin did not blunt the reduction in ejection fraction caused by I/R injury. Linagliptin treatment resulted in a 19-fold increase in plasma GLP-1 levels, and this likely contributed to the reduction in myocardial injury.

Recently, the beneficial effects of DPP-4i, independent of incretin hormone effects, have been shown in a model of uremic cardiomyopathy. Linagliptin treatment of rats with chronic uremic cardiomyopathy lowered the augmented BNP levels and heart tissue fibrosis markers, which likely led to improved cardiac function and structure (34). BNP is one of the off-target peptides implicated in the beneficial effects of DPP-4i in the cardiovasculature. BNP plays a critical role in regulating body fluid homeostasis, has vasodilator effects, and is a marker of HF. Degradation of BNP by DPP-4 lowers guanosine 3’5-cyclic monophosphate (cGMP) levels, resulting in reduced diuresis and natriuresis, as well as reduction in the contribution of BNP to vascular tone. The improvement of cardiac function and fibrosis after linagliptin treatment raises the possibility that elevated BNP in HF is initially the result of stress to the myocardium, and a subsequent decrease in BNP levels with DPP-4i therapy may be indicative of improvement in myocardial stress with concomitant lowering of BNP release. This paradoxical notion that DPP-4i therapy reduces circulating BNP levels was further validated in a recent study reporting elevated circulating DPP-4 activity in patients and rats with HF (45). In that study, the elevated level of BNP associated with experimental HF in rats was reduced in rats on DPP-4i therapy (sitagliptin) in concert with improvement in cardiac function. Taken together, DPP-4i appears to be efficacious in conditions of insulin resistance associated with early diastolic dysfunction in obesity, as well as more advanced cardiomyopathies.

Kidney disease. Obesity and T2DM are associated with progressive loss of renal function. T2DM accounts for 30–40% of CDK and as much as 45% of end-stage renal disease (188). GLP-1Rs are expressed in the kidney, notably in podocytes (77) and the brush border microvilli of proximal tubule cells (38, 165), and DPP-4 is abundantly expressed in capillary endothelial cells (ECs) (170), as well as in the brush border membrane of proximal tubule cells where it assembles with the Na+/H+ exchanger-3 (65, 97). Moreover, renal activity of DPP-4 is elevated in conditions associated with development of diabetic nephropathy, such as in overnutrition/obesity and inflammatory states (195). Recent studies demonstrate that stable GLP-1 analogs and DPP-4i attenuate renal injury in rodent models of type 1 diabetes (102, 115). However, most DPP-4i are excreted through a renal route, thereby raising concern for their use in the presence of renal impairment. In this regard, linagliptin is an exception since it is eliminated mainly through a hepatobiliary route. The renal clearance of linagliptin is very low (<1%), thereby obviating the need for dose adjustment in patients with renal impairment (43, 60, 63, 68, 154). In addition, linagliptin has also been shown to reduce proteinuria in diabetic patients with overt diabetic nephropathy (70, 77). In support of this, we recently observed that linagliptin led to reductions in glomerular injury, proteinuria, and oxidative stress in ZO rats (unpublished data). In another study, linagliptin suppressed activation of transforming growth factor-β, reduced fibronectin expression, and decreased activator protein-1 binding in human kidney proximal tubule cells induced by high glucose (149). These studies highlight the pleiotropic renal effects of DPP-4i beyond glycemic control. Since CKD is associated with CVD, the renal effects of DPP-4i may contribute to improvement in CVD outcomes. In this regard, DPP-4i has been shown to improve cardiac fibrosis, cardiac function in a rodent model of uremic cardiomyopathy (34).

Cellular and Molecular Mechanisms of Action of DPP-4i in the Vasculature

Vascular dysfunction is the critical factor in progression to CVD and CKD and the associated vasculopathies that constitute early derangements mediating progression to end organ damage (5, 8, 137, 138). Vascular dysfunction is independently associated with CVD complications, including stroke, atherosclerosis, and heart and kidney disease (8, 135). In this regard, the development of endothelial dysfunction and vascular stiffness are the early events that contribute to the progression of CVD (6, 32, 186). In the setting of obesity/T2DM, endothelial dysfunction and arterial stiffness may result from multiple factors including insulin resistance, oxidative stress, dyslipidemia, adipose tissue dysfunction (e.g., visceral and perivascular inflammation), and dysfunctional immunity (Fig. 3) (5, 27, 189). Endothelial dysfunction, altered vascular tone, extracellular matrix remodeling, and adventitial dysfunction contribute to arterial stiffness (6, 110); however, arterial stiffness also contributes to endothelial dysfunction (6). These components of the pathophysiology of obesity are modulated by DPP-4i and thus may contribute to CVD protection.

Administration of native GLP-1 and GLP-1 analogs elicit both GLP-1R-mediated responses, as well as effects that are independent of the GLP-1R (15). Some GLP-1R-independent effects in the vasculature have been shown to be mediated by GLP-1(9–36), a stable DPP-4-generated metabolite of active GLP-1(7–36) (15). Specifically, it was shown that GLP-1(9–36) causes vasodilation and increased blood flow in the coronary vasculature, and these effects are mediated by NO-dependent cGMP release, effects that cannot be elicited by the DPP-4-resistant GLP-1 analog exendin-4. The stimulation of NO production

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vascular relaxation. However, edothelin-1 production is unaffected, thereby resulting in an imbalance in vasmediators favoring vascular contraction (Fig. 3).

Increased oxidative stress is largely caused by inappropriate activation of RAAS and accumulation of advanced glycation end products (AGE) (Fig. 3). Oxidative stress in vascular cells can be caused by either cellular reactive oxygen species (ROS) accumulation by NADPH oxidase activation or mitochondrial ROS production (6, 41). However, abnormal activation of NADPH oxidase resulting in increased intracellular ROS levels induces mitochondrial ROS generation, which in turn further activates NADPH oxidase, thereby causing a feed-forward accumulation of ROS. This phenomenon is often termed as “ROS-induced-ROS release” (204, 205). The accumulation of ROS also contributes to endoplasmic reticular stress, leading to insulin resistance and vascular dysfunction (7). Chronic hyperglycemia in diabetes leads to activation of receptor for AGE (RAGE) that transduces the effects of numerous ligands, including posttranslationally glycosylated proteins known as AGEs that are prooxidative, proinflammatory, prothrombotic, and profibrotic (23, 193). RAGE are present in cardiomyocytes, ECs, and vascular smooth muscle cells, as well as in cells recruited to these tissues during stress, such as monocytes, macrophages, and T cells (194).

AGE/RAGE-induced ROS formation induces release of DPP-4 from ECs, inciting a positive feedback loop whereby DPP-4 induces increased expression of RAGE to further exacerbate AGE effects in ECs (84). Furthermore, DPP-4i treatment significantly inhibits the AGE-induced ROS generation in concert with suppression of RAGE, ICAM-1, and plasminogen activator inhibitor-1 gene expression in ECs, thereby providing novel pathways by which DPP-4i suppresses DPP-4 release from the ECs (84). Thus the activation of the AGE/RAGE axis and its interaction with DPP-4 may, in part, explain why ECs and the vasculature are inordinately remodeled and damaged and prone to atherosclerosis and stroke in the setting of diabetes. Accumulating evidence suggests that GLP-1 and DPP-4i may ameliorate the deleterious effects of AGE/RAGE in ECs and cardiac tissue. GLP-1, via the GLP-1R and downstream activation of cAMP, is reported to reduce the expression of RAGE, generation of ROS, and inflammatory markers in HUVECs (85). The DPP-4i vildagliptin reduces RAGE gene and protein expression and suppresses oxidative stress, as well as thrombotic and inflammatory markers in the aorta of type 2 diabetic rats (126). Linagliptin blocks ROS formation in HUVECs generated by the positive feedback loop between AGE/RAGE and DPP-4 (84). Thus DPP-4i could act to protect the endothelium and vasculature in diabetes largely by reducing expression of RAGE and the associated oxidative stress and inflammation.

Microcirculation and vascular recruitment. Recent evidence supports that GLP-1 plays a role in postprandial muscle uptake and use of glucose and insulin (29). In vivo, GLP-1 infusion increases microvascular recruitment that is coupled to muscle insulin uptake and glucose use. These effects are independent of insulin but are mediated by protein kinase A (PKA)-dependent NO release (Fig. 3). Thus it is likely that the effects of DPP-4i on endothelial function may be mediated by GLP-1 and its receptor or direct modulation of DPP-4 activity and pleiotropic actions. Experimental and clinical studies support the notion that GLP-1 is directly beneficial to the vasculature.
[reviewed in Balakumar and Dhanaraj (13), Fadini and Avogaro (52), and Ussher and Drucker (182)].

GLP-2, mesenteric flow, and intestinal function. GLP-2 is a 33-amino-acid peptide substrate of DPP-4 and produced by enteroadocrine cells (48, 74). The plasma levels of GLP-2 are low in the fasting state and rapidly rise after food intake. GLP-2 increases mesenteric blood flow (Fig. 2), facilitates nutrient absorption in the gut, enhances gut permeability, and exerts small bowel cytoprotection (48). The improvement in mesenteric blood flow is secondary to metabolic responses mediated through GLP-2 receptors (19, 20, 48, 74). These findings suggest potential benefits of DPP-4i on mesenteric vascular function.

Senescence and apoptosis. DPP-4i may also serve to improve vascular and EC function by ameliorating EC senescence common in the vasculature of animals and humans with obesity/T2DM. Recently, it was shown that treatment of HUVECs with uncleaved or active GLP-1 (7–36) reduced ROS-induced senescence and DNA damage (144). These antisenescent effects were mediated by GLP-1 binding to the GLP-1R and subsequent downstream signaling through the cAMP/PKA pathway rather than PI3K/Akt. Additionally, GLP-1 induced a PKA-dependent phosphorylation of the transcription factor cAMP responsive element binding protein that promotes cell survival (Fig. 3). In the same study, these results were corroborated in Zucker diabetic fatty rats treated with vildagliptin. Treated rats had elevated GLP-1 levels, and this was associated with a reduction in the augmented levels of vascular cell senescence despite little improvement in glycemia. Whether the observed reduction in EC senescence with vildagliptin is common to all DPP-4i requires further studies. Interestingly, Ban et al. (15) reported that GLP-1 (9–36), the so-called “inactive form” of GLP-1, resulting from enzymatic cleavage of active GLP-1 (GLP-1 [7–36]) by DPP-4, exhibited antiapoptotic effects in EC and cardiomyocytes and that this effect was mediated independently of the GLP-1R. These GLP-1R-independent effects of GLP-1 (9–36) are likely to be minimal with DPP-4i therapy that drastically reduces degradation of GLP-1 (7–36).

Metalloproteinases. MMPs have been implicated in vascular remodeling and disease, including stroke (31, 92, 153). It is possible that MMPs, proteins that play a central role in remodeling extracellular matrix, mediate the antistroke efficacy of linagliptin and exendin-4. At least two studies suggest that GLP-1 may regulate MMP-9 expression and activity. Specifically, GLP-1R agonism is associated with decreased MMP-9 expression and activity in CV tissue (25, 143). We recently observed an increase in active MMP-9 in brain tissue from untreated ZO rats; linagliptin treatment normalized the level of active MMP-9 in the ZO brain (unpublished data). This preliminary observation raises the possibility that the antistroke efficacy of linagliptin could indirectly involve modulation of MMP-9 and that efficacy is likely due to GLP-1 or other DPP-4 substrates present in areas of the brain affected by stroke.

Dyslipidemia. Animal studies demonstrate that DPP-4i (sitagliptin and linagliptin) or GLP-1R agonism (exendin-4) significantly reduce intestinal secretion of TG, cholesterol, and apolipoprotein B-48, suggesting that GLP-1 might directly regulate lipoprotein assembly and secretion in enteroctyes (80, 123). A recent study showed that the DPP-4i allogliptin ameliorates postprandial elevation of TG-rich lipoproteins (lipemia) and endothelial dysfunction in nondiabetic humans following an oral fat load (142), suggesting a potential antiatherogenic role for DPP-4i in humans. Recent clinical studies have reported that DPP-4i such as vildagliptin (124) and sitagliptin (181) improve postprandial atherogenic TG-rich lipoprotein levels in patients with T2DM. Previous studies have shown that DPP-4i such as vildagliptin and sitagliptin decrease postprandial TG, remnant lipoprotein cholesterol and apolipoprotein B-48 levels after a fat-loading test in patients with T2DM (124, 181) and nonobese subjects without diabetes (142). However, the effects of other DPP-4i on postprandial lipid elevation-induced endothelial dysfunction have not been fully evaluated. Although linagliptin exhibited CVD benefits versus a comparator, as reported in a recent meta-analysis of patients with T2DM, it did not reveal a TG lowering effect (90), suggesting other pleitropic effects of linagliptin in the cardiovascular system.

Immune and inflammatory response. Accumulating evidence suggests that a low-grade chronic inflammatory state accompanies obesity, insulin resistance, hypertension, atherosclerosis, and diabetes (164, 202). In these settings, there exists a suite of maladaptive immune responses involving effector and regulatory T cells (Tregs) and polarized macrophages that constitute an inflammatory phenotype, i.e., increased T-helper-17 cells (Th17)/M1 macrophages and reduced Tregs/M2. Tregs are a unique subset of T cells that play an important role in the preservation of self-tolerance and suppression of potentially inflammatory T cells (113). Tregs protect against insulin resistance, in part, by secreting anti-inflammatory cytokines, such as IL-10 and transforming growth factor-β (57, 113, 129, 159). Treg-derived IL-10 suppresses local oxidative stress by inhibiting NADPH oxidase to reduce levels of superoxide (95). On the other hand, Th17 cells secrete IL-17, and these cells can be either cytotoxic or protective depending on the type and site of inflammation (8). In obese mice and in patients with T2DM, Th17 cells are elevated (88).

DPP-4 is expressed widely in CD4 and CD8+ immune cells (37, 109), thus DPP-4i therapy may be beneficial in modulating abnormalities in innate and adaptive immunity (196). Indeed, DPP-4i treatment decreases the accumulation of M1 macrophages and increases levels of M2 macrophages in adipose tissue and in atherosclerotic lesions (51, 168, 171, 178). DPP-4i blunts proliferation and migration of vascular smooth muscle cells from the tunica media into the intima in response to cytokines secreted by damaged ECs to attenuate atherosclerosis. Allogliptin reduced aortic plaques and plaque macrophages in LDL receptor knockout mice fed a high-fat diet (168). Allogliptin also reduced inflammation in this model by inhibiting monocyte activation and chemotaxis. Notably, adipose tissue inflammatory macrophage content (CD11b+ , CD11c+, Ly6C(high)) was reduced concomitantly with upregulation of CD163 positive anti-inflammatory macrophages. Allogliptin showed similar antiatherogenic properties in diabetic ApoE-deficient mice by decreasing the augmented IL-6 and IL-1β expression in atherosclerotic plaques (178). The DPP-4i anagliptin reduced plaque formation and inhibited vascular smooth muscle cell migration in ApoE-deficient mice (51). Moreover, a recent study showed that linagliptin improved insulin sensitivity in a mouse model of diet-induced obesity, and this was accompanied by suppression of adipose tissue macrophage infiltration, suggesting an anti-inflammato-
ry/antiatherogenic role for linagliptin (98), rather than through effects on circulating lipids (90). In addition, linagliptin also attenuated inflammation and improved wound healing in diabetic mice (166). These data suggest that the effect of DPP-4i on ECs and vascular smooth muscle cells may potentially contribute to the inhibition of atherosclerosis. GLP-1 agonists have also been shown to increase Tregs (71), and Tregs promote M2 polarization (113). Thus there is potential to enhance Treg function with DPP-4i therapy. Furthermore, the increase in circulating DPP-4 levels reported in obese patients and animals support the novel use of DPP-4i for suppression of low-grade inflammation and associated tissue insulin resistance as an additional therapeutic benefit to patients with obesity, the metabolic syndrome and T2DM.

Dysfunctional immunity can be mediated, in part, by activation of the RAAS, which is known to modulate macrophage polarization and Th17/Treg balance (75, 94, 120, 164). In this regard, telmisartan, a third generation ARB, improves insulin sensitivity and promotes macrophage polarization to an anti-inflammatory state in visceral adipose tissue (61). Thus co-administration of specific ARBs with DPP-4i could act to potentiate the incretin enhancing and anti-inflammatory effects of DPP-4i to further improve CVD outcomes. Indeed, recent evidence supports the notion that combination DPP-4i/ARB therapy may be emerging as a novel strategy to blunt metabolic and CVD complications of obesity (2, 173, 174).

The role for perivascular adipose tissue (PVAT) dysfunction in promoting vascular inflammation and function is increasingly recognized. Expansion of PVAT and immune cell infiltration that occurs in obesity and T2DM is associated with secretion of inflammatory cytokines and vasoactive hormones (Fig. 3) (177). Moreover, adiponectin from PVAT causes vasorelaxation and its levels are decreased in obesity (30, 118). Therefore, inhibition of DPP-4 in adipose tissue may contribute to vasculoprotection.

**Endothelial repair and angiogenesis: the effects of DPP-4i in modulation of bone-derived endothelial progenitor cells and their mobilization and homing.** The vascular endothelium consists of terminally differentiated ECs that have little capacity to proliferate; as such, there is limited intrinsic reparative capacity. Repair of the injured endothelium is accomplished in large part by recruitment of circulating endothelial progenitor cells (EPCs). The role of endothelial repair is being increasingly recognized as a potential therapeutic target to improve endothelial dysfunction, myocardial ischemic injury, kidney injury, and bone marrow microangiopathy (10, 44, 112, 119). The endothelium of individuals who are obese and diabetic is prone to endothelial injury that can lead to endothelial dysfunction and microvascular and macrovascular complications. Patients with diabetes are reported to be deficient in numbers of circulating EPCs (55), whereas an enriched EPC pool predicts low CV mortality (187). Moreover, EPCs in patients with diabetes exhibit functional impairments because of reduced capacity to proliferate, as well as adhere, migrate, and incorporate into tubular structures (116, 179). In this regard, the chemokine SDF-1α, a known substrate of DPP-4, is emerging as an important regulator of vasculoprotective EPCs, important for endothelial repair and neoangiogenesis. Treatment with DPP-4i blunts the DPP-4-mediated degradation of SDF-1α, thereby enhancing recruitment of EPCs to vascular beds in need of repair or expansion (Fig. 4). Several DPP-4i have been shown to have effects on EPC expansion and recruitment. For example, linagliptin treatment ameliorated cardiac injury and reduced myocardial infarction size and fibrosis in a model of cardiac I/R injury in rats (78). This was associated with increased expression of CD34, c-kit, C-X-C chemokine receptor type 4, and SDF-1α. Since SDF-α is a substrate for DPP-4, these results imply decreased degradation of SDF-1α and increased recruitment of circulating EPCs that promote vascular repair and angiogenesis (170). In support of this, DPP-4 activity in the coronary sinus partly correlated with LV diastolic dysfunction and DPP-4i with vildagliptin reversed LV diastolic dysfunction through an SDF-1α-mediated effect on angiogenesis (170). Moreover, sitagliptin increases SDF-1α levels and circulating EPCs in patients with T2DM, and these effects were considered independent of GLP-1 based on unaltered nitrate/nitrite or plasma glucose levels (54). Sitagliptin also increases SDF-1α and circulating EPCs in a model of hindlimb ischemia (81). Since impaired angiogenesis and rarefaction is associated with obesity/T2DM and contributes to impaired diastolic dysfunction and renal injury, the efficacy of various DPP-4i in promoting SDF-1α-mediated effects may significantly contribute to improved CV outcomes. Incretin signaling has been shown to modulate bone remodeling (28). Therefore, DPP-4i may also improve bone repair by their effects on EPCs and SDF-1α, in addition to their actions on bone cells (28). Furthermore, DPP-4i may act to increase EPC numbers by potentiating the bone marrow mobilizing effects of granulocyte-colony stimulating factor-1 (53, 54). Moreover, ARBs have been shown to increase EPCs and improve vascular repair (127). These findings suggest that the combination of
ARBs and DPP-4i have potential synergistic benefits on bone health and EPCs. Thus DPP-4i acts to improve CV outcomes by affecting improvements in endothelial function,ameliorating EC senescence and apoptosis, and promoting blood vessel repair (Fig. 4).

Conclusions

DPP-4i are potent, highly selective inhibitors of DPP-4 approved for treatment of T2DM as either monotherapy or in combination with conventional diabetes therapies. To date, most DPP-4i have good safety and tolerability profiles with low incidence of adverse events. The abnormal increase in circulating DPP-4 levels in individuals who are obese and diabetic underscores the significance of targeting DPP-4 as a therapeutic strategy. The advantage of DPP-4i stems from its multiple beneficial actions that improve endothelial function to protect the vasculature. Preclinical evidence suggests that DPP-4i confers vasculoprotection and the potential vasculo-protective properties could reduce the risk for further development of the multiple comorbidities associated with obesity/T2DM, including hypertension and heart and kidney disease. The efficacy of DPP-4i is due, in part, to effects unrelated to improved glycemia. Potential benefits in the vasculature include increased NO generation, reductions in oxidative stress and inflammatory cell infiltration, and increased recruitment of EPCs, effects that collectively act to repair the injured endothelium and improve endothelial function (Fig. 1). Nonetheless, pending results from ongoing CV outcomes trials will be critical to further evaluate whether specific or collective benefits of DPP-4i therapy in the vasculature reduce CVD risk or mortality in T2DM, as well as elucidate potential risks not yet discovered. Further study is also needed to determine whether DPP-4i induce favorable alterations in immune and inflammatory responses that play a causative role in development and progression of vascular dysfunction and the associated CVD and CKD. Coadministration of DPP-4i and ARBs, but not ACEi, may be an attractive and safe therapeutic combination to treat patients with T2DM, especially those with hypertension.

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