The Effects of Dipeptidyl Peptidase-4 Inhibition on Microvascular Diabetes Complications

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REVIEW

We performed a review of the literature to determine whether the dipeptidyl peptidase-4 inhibitors (DPP4-I) may have the capability to directly and positively influence diabetic microvascular complications. The literature was scanned to identify experimental and clinical evidence that DPP4-I can ameliorate diabetic microangiopathy. We retrieved articles published between 1 January 1980 and 1 March 2014 in English-language peer-reviewed journals using the following terms: ("diabetes" OR "diabetic") AND ("retinopathy" OR "retinal" OR "nephropathy" OR "renal" OR "albuminuria" OR "microalbuminuria" OR "neuropathy" OR "ulcer" OR "wound" OR "bone marrow"); ("dipeptidyl peptidase-4" OR "dipeptidyl peptidase-IV" OR "DPP-4" OR "DPP-IV"); and ("inhibition" OR "inhibitor"). Experimentally, DPP4-I appears to improve inflammation, endothelial function, blood pressure, lipid metabolism, and bone marrow function. Several experimental studies report direct potential beneficial effects of DPP4-I on all microvascular diabetes-related complications. These drugs have the ability to act either directly or indirectly via improved glucose control, GLP-1 bioavailability, and modifying nonincretin substrates. Although preliminary clinical data support that DPP4-I therapy can protect from microangiopathy, insufficient evidence is available to conclude that this class of drugs directly prevents or decreases microangiopathy in humans independently from improved glucose control. Experimental findings and preliminary clinical data suggest that DPP4-I, in addition to improving metabolic control, have the potential to interfere with the onset and progression of diabetic microangiopathy. Further evidence is needed to confirm these effects in patients with diabetes.

Diabetes increases the incidence of cardiovascular disease (CVD) (1), but the role of hyperglycemia in the pathogenesis of CVD is still under debate. Recent large trials have shown that, at least for the duration of the trials, glucose lowering has modest or neutral effects on CVD in people with type 2 diabetes (T2D) (2–5). On the other hand, the relationship between hyperglycemia and microvascular outcomes is very strong, as high glucose promotes activation, dysfunction, and apoptosis of vascular and nonvascular cells (6). Importantly, diabetic microvascular changes affect the retina, kidney, and nerves, but they can also be detected in other organs, such as the heart (7,8). For instance, we have shown that acute hyperglycemia in T2D significantly alters myocardial microvascular perfusion (9). Although the analogy between site-specific microvascular complications is difficult to assess, the association between retinal disease and nonretinal consequences of diabetes is supported by a fairly relevant amount of clinical and experimental data (10). For instance, the presence of diabetic

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retinopathy is associated with a two- to threefold higher risk of incident fatal and nonfatal coronary heart disease, even after adjustment for traditional risk factors, and up to 25-fold higher prevalence of lower limb amputation (11,12). A relationship between retinopathy and the extent of coronary artery calcium was observed (13), and microangiopathy is independently associated with presence, severity, and composition of carotid atherosclerosis (14). These data suggest the existence of common, yet unknown, pathogenic mechanisms and mutual relationships between microvascular disease and cardiovascular risk. Diabetic nephropathy is another strong CVD predictor. Microalbuminuria is itself recognized as an independent determinant of mortality and CVD in both the general and diabetic populations (15,16), and chronic kidney disease, starting from stage 1, is well documented as an amplifier of the cardiovascular risk (17). More recently, diabetes has also been shown to induce microangiopathy in the bone marrow (BM) of mice and humans (18,19). By providing regenerative vascular stem/progenitor cells, the BM acts as a central housekeeper of cardiovascular health, whereas BM microangiopathy may impair cardiovascular homeostasis (20). Large randomized prospective studies have shown that, despite a neutral effect on macrovascular disease, HbA_{1c} control significantly reduces microvascular end points (2,21–24). However, either the short duration of these studies or the adverse effects of glucose-lowering agents do not allow us to (dis)prove the causal relationship between the prevention of microvascular complications and the subsequent improvements in cardiovascular outcomes.

So-called incretinergic therapies have boosted enthusiasm in the treatment of patients with T2D since both GLP-1 receptor agonists (GLP-1RA) and dipeptidyl peptidase-4 (DPP-4; also termed CD26) inhibitors (DPP4-I) have been shown to exert significant glucose-lowering effect without inducing weight gain and with risk of hypoglycemia comparable to placebo (in monotherapy and when associated with metformin) and lower than that associated with sulfonylureas (25,26). Furthermore, the literature offers lots of data showing positive effects on vascular biology beyond glucose lowering (27). Many efforts, including preclinical studies, pooled analysis of phase III clinical trials, and randomized controlled trials, have been devoted to test the potential cardiovascular benefits of DPP4-I, while their impact on microangiopathy seems to be relatively neglected. So far, randomized controlled trials do not support cardiovascular protective effects of DPP4-I (23,28), but studies with longer follow-up are likely needed to allow the effect of good glycemic control achieved with DPP4-I to translate into improved cardiovascular outcomes (3). We hypothesize that the effects of DPP4-I on microangiopathy may be one key element in this time-dependent effect. In this review, the role of DPP4-I on diabetes-related microvascular complications will be considered and discussed from both experimental and clinical perspectives.

DPP-4 STRUCTURE AND FUNCTION

DPP-4 is a highly conserved peptidase with high selectivity for peptides with a proline or alanine at the second NH_2-terminal position. The CD26 gene encodes a type II transmembrane protein of 766 amino acids, which is anchored to the lipid bilayer by a single hydrophobic segment located at the N-terminus and has a short cytoplasmic tail of six amino acids (29). The extracellular part of CD26 contains a glycosylation domain, a cysteine-rich domain, and a catalytic domain. Furthermore, CD26 presents interactions with the extracellular matrix, namely, fibronectin and collagen. In 1993, CD26 was identified as the binding protein for adenosine deaminase (30), the deficiency of which causes severe impairment of cellular and humoral immunity. Formation of the complex between adenosine deaminase and CD26 preserves the individual enzymatic activities of both molecules, and a model for immunoregulation proposes that CD26 modulates the concentration of local extracellular adenosine, which provides negative signals to T cells (31). CD26 is strongly expressed on epithelial cells (kidney proximal tubulus, intestine, bile duct), on endothelial cells, as well as on leukocytes. The soluble form of CD26 (sCD26 or plasma DPP-4) lacks the intracellular tail and transmembrane regions. A consistent number of proteins have a penultimate alanine, proline, or serine at the N-terminus: these proteins act on many different cell types, tissues, and organ systems that may be affected by DPP-4 (32). Beside incretin hormones, these substrates include neuropeptides, cytokines, growth factors, and chemokines. Stromal cell-derived factor 1α (SDF-1α) and 1β (CXCL12), macrophage-derived chemokine (CCL22), interferon-inducible T-cell α-chemoattractant (CXCL11), granulocyte chemotactic protein 2 (CXCL6), and Groβ (CXCL2) are some relevant known substrates for proteolytic modification by DPP-4. Neuropeptide Y (NPY) and peptide YY belong to the pancreatic polypeptide family involved in neuroendocrine control of feeding-associated processes (33). Peptide YY is secreted in response to neuronal and humoral factors, as well as nutrients, and has vasoconstrictive properties. The varieties of physiologic functions that can be theoretically modified by DPP-4 substrates indicate that DPP4-I can have important effects well beyond incretins and glucose control.

Despite a common mechanism of action, there is a significant heterogeneity in the pharmacokinetic of different DPP4-I: they show differences in half-life, bioavailability, metabolism, and excretion route. Some DPP4-I act through competitive enzymatic inhibition (sitagliptin and alogliptin), while others are substrate-enzyme blockers (saxagliptin and vildagliptin) (34). DPP4-I might also differ in their protective effect against microangiopathy, especially in terms of nephroprotection, according to their route of elimination: less than 5% of linagliptin is excreted through the kidney, while all other DPP4-I are mostly excreted through the renal route. Whether this different route of excretion translates into a direct protective effect on kidney function in people with diabetes is presently unknown.

Literature Search Strategy

To review the current knowledge on DPP4-I in diabetic microangiopathy, potentially relevant articles were retrieved from PubMed, ISI Web of Knowledge, and Scopus using the following combination of search terms: ("diabetes" OR "diabetic") AND ("retinopathy" OR "retinal" OR "nephropathy" OR "renal" OR "albuminuria" OR "microalbuminuria" OR "neuropathy" OR "ulcer" OR "wound" OR "bone marrow"); ("dipeptidyl peptidase-4" OR "dipeptidyl peptidase-IV" OR "DPP-4" OR "DPP-IV"); and ("inhibition"

OR "inhibitor"). As of 3 June 2014, this search retrieved 160 articles for review. The title, abstract, and key words were used to screen items unrelated to the topic of interest. Cross-reference and citations of other relevant items were checked in articles selected for further analysis.

Effects of DPP4-I on Endothelial Function and Risk Factors for Endothelial Dysfunction

Endothelial cells exposed to high glucose exhibit enhanced DPP-4 activity (35); conversely, DPP4-I is associated with an increased biological activity of nitric oxide (36,37). DPP4-I also induced a significant reduction of CD40 (38), intracellular adhesion molecule 1 (ICAM-1), and transendothelial migration of circulating mononuclear cells (39). In spontaneously hypertensive rats, DPP4-I improves endothelium-dependent vasodilatation of renal arteries, renormalizes renal blood flow, and reduces systolic blood pressure (37). A pivotal contributor to diabetic vascular damage is determined by the overproduction of advanced glycation end products (AGEs) that bind to their specific receptors (receptors for AGEs [RAGEs]), inducing oxidative stress, inflammation, and thrombogenicity. In a model of OLETF rats, Matsui et al. (40) showed that vildagliptin treatment significantly reduced expression of RAGE, components of NADPH oxidase (gp91phox and p22phox), and markers of oxidative stress in the thoracic aorta. Ishibashi et al. (41) showed that sitagliptin in combination with GLP-1 completely blocked the AGE-induced increase in RAGE mRNA and protein, thus preventing reactive oxygen species generation and endothelial nitric oxide synthase (eNOS) downregulation. Two weeks of vildagliptin treatment in type 1 diabetic (T1D) rats reduced oxidative stress and suppressed ICAM-1, transforming growth factor β, and plasminogen activator inhibitor 1 gene expression (42). Recently Zeng et al. (43) showed that mice treated with sitagliptin developed smaller atherosclerotic plaques than controls, had reduced collagen content in plaques, and reduced the expression of MCP-1 and interleukin (IL)-6 in the aorta. The authors suggest that these effects of sitagliptin may be carried out via regulation of the AMPK and mitogen-activated protein kinase pathways. In humans, we have shown that DPP4-I with sitagliptin or saxagliptin in T2D is able to increase endothelial progenitor cell (EPC) levels (44) and function (45). Some authors have shown an improvement of endothelial function (46–49), which has been, however, confuted by others (50).

Clinical studies have demonstrated some improvement in lipid profile by DPP4-I–based therapies. In a meta-analysis of available trials, Monami et al. (51) reported that treatment with DPP4-I is associated with a significant reduction in total cholesterol and triglycerides without significantly affecting HDL. In a more recent review, van Genugten et al. (52) report a fairly positive effect of DPP4-I on plasma lipids, particularly for sitagliptin on HDL cholesterol and for vildagliptin on total cholesterol. Eventually, in nondiabetic subjects, Noda et al. (47) correlated alogliptin-mediated improvement in endothelial function to its ability to suppress the postprandial elevation of triglycerides, apolipoprotein B48, and remnant lipoprotein cholesterol.

The effects of DPP4-I on blood pressure appear more complex and less clear. DPP-4 converts the NPY(1-36), released by sympathetic renal fibers and agonist of Y1 receptor, to NPY(3-36), the selective agonist of Y2 receptor (53). Since Y1 receptors potentiate renovascular response to angiotensin II (AT-II), it has been postulated that DPP4-I might sustain NPY(1-36) capacity to increase the hypertensive response to AT-II. Animal studies have shown the DPP4-I improved endothelium-dependent relaxation in renal arteries, restored renal blood flow, and reduced systolic blood pressure in spontaneously hypertensive rats by increasing cAMP level and eNOS (49). Approximately 70% of excreted Na^+ is reabsorbed in the proximal tubule, via a Na^+/H^+ exchanger 3: DPP-4 forms a complex with Na^+/H^+ exchanger 3 at the level of the brush membrane (54). DPP4 inhibition with sitagliptin administration may interfere with Na^+ resorption mechanism, significantly increasing natriuresis, thereby reducing blood pressure levels (55,56). Another DPP-4 substrate that can play an important role in blood pressure regulation is the brain-derived natriuretic peptide (BNP). DPP-4 converts the active form of BNP (1–33) into a form inactive on natriuresis but still active on cyclic guanosine monophosphate production in cardiomyocytes (57). Therefore, DPP4-I administration can potentially decrease blood pressure by two distinct mechanisms: one at the renal level, by inhibiting sodium/hydrogen exchange, and the other at cardiac level, by inhibiting BNP degradation. Whether the effect of DPP4-I on BNP has any role on endothelial function in humans is presently unknown.

Clinical data demonstrate a modest blood pressure reducing effect of DPP4-I (52,58,59). This contrast with the well-known blood pressure–lowering effects of incretinergic therapy with GLP-1RA (60), which is mediated by atrial natriuretic peptide secretion (61). Rather, owing to complex interactions between concomitant ACE and DPP4-I, blood pressure regulators that are sequential substrates of these two enzymes may mediate unexpected effects in particular clinical settings. For instance, it has been shown that, in healthy volunteers, substance P increases sympathetic activity when ACE and DPP-4 are both inhibited (62). Such effect may limit blood pressure control obtained with maximal ACE inhibition (63). However, it should be emphasized that very few clinical studies included reduction of blood pressure as a primary or prespecified end point.

Inflammation in dysmetabolic conditions is the result of expanded fat mass, and it plays a causative role in inducing insulin resistance, as well as atherosclerotic plaque instability. DPP-4 plays an important role in the immune and inflammatory responses (64). Both obese humans and rodents demonstrated increased levels of DPP-4 expression in dendritic cell/macrophage populations from visceral adipose tissue (65). Lamers et al. (66) have suggested DPP-4 can be considered a new adipokine, released by human adipocytes: DPP-4 expression is particularly high in visceral fat of obese subjects and correlates with all metabolic syndrome components. It is therefore intuitive that DPP4-I has the potential to suppress such a proinflammatory state. In diabetic Zucker rats, the chronic administration of sitagliptin reduced C-reactive protein and IL-1β levels, along with improvement of oxidative stress (67). Sitagliptin, in the diet-induced obesity mouse model, reduced the proinflammatory milieu, macrophage infiltration, and gene expression of MCP-1, IL-6, IL-12 (p40),

and IL-12 in the adipose tissue (p35) (68). Similar anti-inflammatory effects of DPP-4 have also been demonstrated in the atherosclerotic plaque. Alogliptin treatment, in ApoE$^{-/-}$ diabetic mice, induced a significant reduction of atherosclerotic lesions and a concomitant reduction of IL-6 and IL-1β (69). Vittone et al. (70) showed that in ApoE$^{-/-}$ mice, sitagliptin reduced plaque inflammation and increased plaque stability, potentially by GLP-1–mediated inhibition of chemokine-induced monocyte migration and macrophage matrix metalloproteinase 9 release. Interestingly, it was shown that linagliptin administered in a rat model of sepsis ameliorated lipopolysaccharide-induced endothelial dysfunction in addition to reduced aortic infiltration with inflammatory cells (71).

In humans, Rizzo et al. (72) observed that vildagliptin determined reductions in nitrotyrosine, IL-6, and IL-18 concentrations that were correlated with lessened glycemic variability. This might have a potential benefit of preventing atherosclerosis progression in patients with T2D (73). In another study, sitagliptin induced a significant reduction of proinflammatory cytokines, TNF-α, endotoxin receptor, Toll-like receptors 4 and 2, nuclear factor-kB, and C-reactive protein and IL-6 concentrations (74). Satoh-Asahara et al. (75) showed that sitagliptin 50 mg q.i.d. for 3 months decreased serum levels of amyloid A–LDL, C-reactive protein, and TNF-α. In conclusion, both experimental and human studies consistently report that DPP4-I provides endothelial protection and blunts inflammation. While the link between inflammation and atherogenesis is well known (76), it should be noted that inflammation also promotes development and progression of diabetic microangiopathy, including nephropathy, retinopathy, and neuropathy (77–79).

DPP4-I and the Kidney

Experimental Studies

Since the discovery of DPP-4 as an adenosine deaminase binding protein (80), the expression of DPP-4 has been considered a marker of renal injury, including diabetic nephropathy (81–83). The relationship between DPP-4 activity and kidney function is complex, and available studies frequently report conflicting results. Tofovic et al. (84) were among the first to underline this complexity, also in the light of the pleiotropic effect of DPP-4. They showed that inhibition of DPP-4 prevents the catabolism of NPY(1-36) and thereby increases the effects of NPY(1-36) released from renal sympathetic nerves on Y1 receptors, thus leading to an enhancement of the renovascular effects of AT-II (85). Along this line, Tofovic et al. (84) showed that sitagliptin enhances renovascular responses to AT-II in SHR rats; they also demonstrated that this effect persists in rats with diabetic nephropathy and metabolic syndrome. Kirino et al. (86) assessed renal function in F344/DuCrlCrlj rats, a substrain of the inbred Fischer 344 strain lacking DPP-4 enzyme activity. Interestingly, they showed that DPP-4–deficient rats were relatively resistant to developing streptozotocin (STZ) diabetes, but once diabetic, they were more susceptible to reduction of glomerular filtration rate. Conversely, Mega et al. (87) assessed the effect of chronic low-dose sitagliptin on renal lesions in a T2D rat model of diabetic nephropathy. Sitagliptin ameliorated renal lesions, including glomerular, tubulointerstitial, and vascular lesions. Whether these effects were direct or dependent from glucose reduction was not ascertained. Consistent with these observations, another study reported that sitagliptin decreased IL-1β and TNF-α levels and prevented the increase of BAX/Bcl-2 ratio, Bid protein levels, and TUNEL--positive cells. Such data indicate protective effects against inflammation and apoptosis in the kidney (88). In a study addressing prevention of renal damage, pretreatment of diabetic animals with sitagliptin was associated with normal serum creatinine, blood urea nitrogen, and expression of tissue injury markers following renal ischemia/reperfusion injury, while leading to normalization of blood glucose to control levels (89). The beneficial effects of DPP4-I have been observed in STZ eNOS$^{-/-}$ mice, a rodent model of human diabetic nephropathy: the coadministration of linagliptin and the angiotensin receptor blocker telmisartan was associated with a marked reduction in albuminuria, though telmisartan or linagliptin alone did not significantly lower this parameter (90). In another study performed in STZ diabetic rats, linagliptin reduced AGE and RAGE levels, quenched oxidative stress, improved albuminuria, and ameliorated histological features of glomerulopathy (91), lending support to the protective effects of linagliptin against diabetic nephropathy. Liu et al. (92) assessed whether vildagliptin had a renoprotective activity in STZ-induced diabetic rats. Diabetic and nondiabetic rats were treated with oral vildagliptin or placebo for 24 weeks, and renal injury was observed by light and electron microscopy. Diabetic rats exhibited marked polyuria, increased urinary albumin and protein excretion, high serum creatinine and blood urea nitrogen levels, enhanced albumin/creatinine ratio (ACR), and decreased creatinine clearance at weeks 12 and 24. Repeated treatments with vildagliptin significantly reduced diabetic albuminuria, proteinuria, and serum creatinine in diabetic rats at week 12. The fractional mesangial area and extent of segmental glomerulosclerosis were significantly higher in the untreated diabetic group compared with the nondiabetic groups. Treatment with vildagliptin significantly lowered the fractional mesangial area and reduced the glomerular sclerosis indexes in diabetic rats. Furthermore, vildagliptin reduced interstitial expansion of diabetic rats by 33%. A more recent study by Vavrinec et al. (93) showed that vildagliptin, without affecting plasma glucose levels or proteinuria, was able to decrease glomerulosclerosis and restore myogenic arteriolar constriction to normal levels, possibly due to reduced oxidative stress. This series of experimental studies shows that DPP4-I at the kidney level may promote both negative and positive effects, with most data pointing to protective effects of DPP4-I on kidney function. Whether these effects are direct or partially mediated by changing glucose concentration warrants further scrutiny, but results obtained in models of T1D seem to support a direct effect.

Clinical Studies

Diabetic nephropathy in humans is the consequence of glomerular, tubular, vascular, and interstitial structural abnormalities and dysfunctions. The main stem of preservation of renal function is glucose-lowering strategies. In several trials, the reduction in HbA_{1c} has been paralleled by an improvement in renal function metrics. In the UK Prospective Diabetes Study (UKPDS) trial, each 1% reduction in updated mean HbA_{1c} was associated with reductions in risk of 37% for microvascular complications (94); specifically, the risk reduction for

albuminuria was 34%. An even more remarkable effect (54%) was observed in the Diabetes Control and Complications Trial (DCCT) (95). In the Veterans Affairs Diabetes Trial (VADT), any worsening of albumin excretion as well as the progression to macroalbuminuria was lower in the intensively treated group than in the standard-treated group (4). In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, a reduction in microalbuminuria (−9%) and in macroalbuminuria (−29%) was observed in the intensively treated group (5). Similar effects were reported in the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) trial, with a 21% reduction in new or worsening nephropathy in those patients randomized to intensive treatment (2). Therefore, a key question is whether DPP4-I are able to improve renal metrics beyond their antihyperglycemic effect.

Few studies have been devoted to directly assessing the effects of DPP4-I on renal functional measures. Hattori (96) investigated the effect of sitagliptin (50 mg/day) on albuminuria in patients with T2D. Sitagliptin significantly lowered both systolic and diastolic blood pressures, fasting blood glucose and postprandial blood glucose, HbA_{1c}, and glycated albumin at 3 and 6 months. They showed that the urinary ACR did not change in the 6 months before sitagliptin treatment and decreased significantly in the 6 months after sitagliptin treatment. They hypothesized that sitagliptin reduces albuminuria without lowering the estimated glomerular filtration rate, most likely depending on blood glucose reduction and improved blood pressure control. In a crossover study with two DPP4-I, sitagliptin and alogliptin, in 12 T2D patients with microalbuminuria, taking angiotensin receptor blockers, it was found that alogliptin reduced urinary albumin levels (97). Tani et al. (98) evaluated the effects of the DPP4-I vildagliptin on atherogenic LDL heterogeneity and albuminuria in diabetic subjects. After 8 weeks of treatment, the ACR decreased significantly by ~45%. Recently, Groop et al. (99), in a pooled analysis of four studies, identified 217 subjects with T2D and prevalent albuminuria while receiving stable doses of renin-angiotensin-aldosterone system inhibitors. Participants were randomized to either linagliptin 5 mg/day or placebo. The primary end point was the percentage of change in geometric mean ACR from baseline to week 24. ACR at week 24 was reduced by 32% with linagliptin compared with 6% with placebo. Either HbA_{1c} or systolic blood pressure, at baseline or after treatment, did not influence the albuminuria-lowering effect of linagliptin. However, the mechanisms accounting for the direct beneficial effect of linagliptin on album excretion remain unclear (100).

Several trials have been performed to determine safety of DPP4-I in diabetic patients with various degrees of renal impairment (Table 1). This is important because episodes of renal failure have been reported in patients using this category of glucose-lowering agents. However, it should be emphasized that in few studies, a direct effect of DPP4-I has been assessed on kidney function metrics, such as estimated glomerular filtration rate and microalbuminuria. Noteworthy, in the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients With Diabetes Mellitus (SAVOR) trial, patients treated with saxagliptin were significantly more likely than patients receiving placebo to have an improved ACR and less likely to have a worsening ratio (23). At the end of the trial, 13.3% of those receiving saxagliptin had a worse ratio ($<$3.4, $\geq$3.4 to $\leq$33.9, or $>$33.9 mg/mmol ratio categories, respectively) versus 15.9% of those on placebo. Among those on saxagliptin, 10.7% had improved ratio versus 8.7% among those on placebo. These data suggest a protection of the DPP4-I on albumin excretion rate. Though the end-of-trial difference in HbA_{1c} between saxagliptin- and placebo-treated patients was small, it remains unclear whether these effects are determined by glucose control itself or a direct effect of DPP4-I, as suggested by experimental studies.

DPP4-I and Diabetic Retinopathy

Intensive glucose control has been shown to provide beneficial effects on retinopathy in both T1D (101) and T2D (21,102). Few experimental studies have assessed the effect of DPP4-I on diabetic retinopathy. In Zucker diabetic fatty rats, Gonçalves et al. (103) assessed the efficacy of sitagliptin in preventing glucose-mediated damage on the blood-retinal barrier. They determined the content and/or distribution of tight junction proteins occludin and claudin 5, the nitrotyrosine residues, and retinal cells apoptosis as well as EPC adhesion to retinal vessels. They found that treatment with sitagliptin prevented the changes in the endothelial subcellular distribution of the tight junction proteins induced by diabetes. Sitagliptin also decreased the nitrosative stress, inflammatory state, and apoptosis in diabetic retinas. Diabetic animals showed decreased circulating EPC levels and EPC adhesiveness to the retinal vessels. Sitagliptin allowed a recovery of the number of EPCs present in the bloodstream to levels similar to their number in controls and increased their adhesive capacity. In another study, Maeda et al. (104) showed that vildagliptin significantly increased retinal gene expression of vascular endothelial growth factor, intercellular adhesion molecule 1, plasminogen activator inhibitor 1, and pigment epithelium-derived factor. Gonçalves et al. (105) have also shown that in the retina of STZ-induced T1D rats, sitagliptin prevented the increase in blood-retinal barrier permeability and inhibited the changes in immunoreactivity and endothelial subcellular distribution of occludin, claudin 5, and zonula occludens 1 proteins induced by diabetes. Furthermore, sitagliptin decreased the retinal inflammatory state and neuronal apoptosis, thus indicating a direct protective effect on diabetic retinal cells.

Regarding clinical data, in a recent small double-blind, placebo-controlled, crossover trial in 50 T2D patients without retinopathy, Ott et al. (106) found that 6 weeks of saxagliptin treatment significantly reduced retinal capillary blood flow and improved vasodilation. However, to the best of our knowledge, no study has so far evaluated the effects of DPP4-I on retinopathy end points in diabetic patients.

DPP4-I and Neuropathy

Only three experimental studies are available on the effect of DPP4-I on diabetic neuropathy. In the first report, the authors investigated the GLP-1 pathway effect on peripheral nerves using vildagliptin in STZ-induced diabetic rats (107). They showed that daily administration of vildagliptin protected from nerve fiber loss compared with

Table 1—Summary of the effects of DPP4-I on microvascular end points at the various sites of diabetes complications

	Experimental		Clinical	
Complication	Model	Effects	Drug and patients	End point
Nephropathy	STZ diabetic DPP-4–deficient (F344/DuCrlCrlj) rats (86)	↓ GFR	Sitagliptin in 36 T2D patients (96)	↓ hs-CRP, ICAM-1 ↓ Albuminuria
	Sitagliptin in Zucker diabetic rats (87)	↓ Glomerular, tubulointerstitial, and vascular lesions	Alogliptin vs. sitagliptin (crossover) in 12 T2D patients (97)	↓ Albuminuria
	Sitagliptin in Zucker diabetic fatty rats (88)	↓ Tubulointerstitial and glomerular lesions ↓ Apoptosis	Vildagliptin in 47 T2D patients (98)	↓ Albuminuria
	Sitagliptin in ischemia reperfusion injury in nicotinamide/STZ diabetic rats (89)	↑ GFR ↓ Oxidative stress ↓ Tissue damage	Linagliptin vs. placebo in 217 T2D patients with micro-/macroalbuminuria (pooled analysis) (99)	↓ Albuminuria
	Linagliptin + telmisartan in STZ eNOS$^{-/-}$ mice (90)	↓ Albuminuria ↓ Glomerulosclerosis	Saxagliptin vs. placebo in >16,000 T2D patients (23)	↓ Microalbuminuria
	Linagliptin in STZ diabetic rats (91)	↓ AGE and RAGE ↓ Oxidative stress ↓ Albuminuria ↓ Glomerulosclerosis		
	Vildagliptin in STZ diabetic rats (92)	↑ GFR ↓ Albuminuria ↓ Glomerulosclerosis ↓ Interstitial fibrosis		
	Vildagliptin in Zucker diabetic fatty rats (93)	↓ Glomerulosclerosis ↑ Arteriolar function ↓ Oxidative stress		
Retinopathy	Sitagliptin in Zucker diabetic fatty rats (103)	↓ Nitrosative stress, inflammation, apoptosis ↓ Blood-retinal barrier changes ↑ EPCs	Saxagliptin vs. placebo in 50 T2D patients without retinopathy (106)	↓ Blood flow ↑ Vasodilation
	Sitagliptin in STZ diabetic rats (105)	↓ Permeability ↓ Blood-retinal barrier changes ↓ Inflammation, apoptosis		
	Vildagliptin in OLETF T2D rats (104)	↓ VEGF, ICAM-1, PAI-1, and PEGF		
Neuropathy	Vildagliptin in STZ diabetic rats (106) PKF275-055 in STZ diabetic rats (108)	↓ Nerve fiber loss ↑ Na^+/K^+-ATPase activity ↑ Nerve conduction velocity ↑ Mechanical and thermal sensitivity	No data available so far	
	Sitagliptin in nicotinamide/STZ T2D rats (109)	↑ Strength and paw function ↓ Nerve cell loss		
Foot ulcers	Linagliptin in *ob/ob* T2D mice (112)	↑ Epithelialization ↑ Myofibroblasts ↓ Inflammation	Vildagliptin vs. placebo in 106 diabetic patients with ulcers (113)	↑ Granulation tissue ↑ Capillary density ↑ HIF-1α and VEGF ↓ Nitrotyrosines ↓ Proteasome activity

Experimental and clinical data are reported separately. GFR, glomerular filtration rate; HIF, hypoxia inducible factor; PAI, plasminogen activator inhibitor; PEGF, placenta-derived growth factor; VEGF, vascular endothelial growth factor.

untreated rats, and they also observed a significantly lower decrease of intraepidermal nerve fiber density. In a second study, the beneficial effects of PKF275–055, a selective DPP4-I, were tested in STZ-induced diabetic peripheral neuropathy (108). It was shown that this drug partially counteracted the nerve conduction velocity deficit observed in untreated diabetic rats but did not improve mechanical and thermal sensitivity. When used in a therapeutic setting, PKF275–055 treatment restored mechanical sensitivity thresholds by ~50% and progressively improved the alteration in thermal responsiveness. Finally, in rats with nicotinamide-/STZ-induced diabetes, Sharma et al. (109) observed that sitagliptin and sitagliptin combined with metformin or amitriptyline resulted in neural protection and reversed the alteration of biochemical parameters in diabetic rats. Given the paucity of treatment strategies available to reverse the clinical features of diabetic neuropathy, these experimental evidences on the protective effects of DPP4-I are promising and deserve future attention.

DPP4-I and Diabetic Foot Ulcers

Delayed wound healing in diabetes is a major source of morbidity and mortality. It results from the combination of vasculopathy and neuropathy, and often leads to minor and major amputations

(110). Many diabetic patients with ischemic foot ulcers are not amenable to surgical revascularization of lower limb arteries because of multiple distal stenosis. In addition, microangiopathy is a major contributor to the shortage of oxygen and nutrient supply to the granulation tissue, thus contributing to delayed healing (111). In this setting, it is of paramount importance to devise therapeutic strategies to restore the structure and function of the epithelium and granulation tissue to aid wound healing in diabetic patients. For the possible improvement in microvascular outcomes obtained with DPP4-I as outlined above, these drugs are novel candidates for the medical treatment of diabetic foot ulcers.

Experimentally, it was shown that DPP-4 expression and activity are increased in the wounded skin of diabetic obese mice (112). In turn, excess DPP-4 activity is supposed to degrade the chemokine and angiocrine factor SDF-1α (CXCL12), thus impairing vascularization and growth of the granulation tissue. Consistently, DPP4-I with linagliptin resulted in accelerated wound healing in diabetic obese mice compared with untreated mice. This was associated with improved reepithelialization, reduced inflammation, and enhanced the formation of myofibroblasts, all features of a healthier granulation tissue (112).

As a clinical counterpart of these experimental observations, it was found that treatment with a DPP4-I for just 12 weeks improved healing features in T2D patients with chronic nonhealing foot ulcers, improving nitrosative stress, response to hypoxia, and capillary density (113). Although the mechanisms that translate DPP4-I into such a strong improvement in granulation tissue structure and function in vivo remain to be completely understood, these preliminary data are promising and, if confirmed, would enrich our therapeutic armamentarium to accelerate wound healing in diabetes.

DPP4-I and the Diabetic BM

In addition to being the major source of hematopoietic cells in the adult organism, the BM is a reservoir of stem/progenitor cells involved in endothelial repair and angiogenesis (20). EPCs, together with other progenitor cell phenotypes, are reduced in diabetes, especially in the presence of any macro- or microvascular complication (114). Nowadays, shortage of EPCs is considered a contributor to the development of diabetes complications. Therefore, investigations into the mechanisms that impair EPC in diabetes are of great interest to devise new endogenous regenerative therapeutic strategies (115). Experimental modeling suggests that the low circulating EPC level in diabetes is attributable to impaired mobilization from the BM. Indeed, in the last years, it has been recognized that the BM is a novel and hitherto neglected site of diabetic microangiopathy (116). Features of the diabetic BM in rodents and humans include autonomic neuropathy (117,118); rarefaction of capillaries, arterioles, and sinusoids; increased microvascular permeability; excess oxidative stress; and relocation of stem cells relative to altered oxygen gradients across an extensively remodeled BM niche (18,19,119). As a consequence of these profound structural and functional abnormalities, BM stem cells show reduced survival and altered responsiveness to mobilizing agents, a condition now deemed as "diabetic stem cell mobilopathy" (120). Indeed, in humans and animals, BM stem cell mobilization is impaired by diabetes (121,122). Importantly, DPP-4 seems to play a major role in the regulation of stem cell trafficking and its role in the diabetic BM dysfunction has been shown recently (123). Indeed, DPP-4 proteolytic activity determines the systemic and local concentrations of the chemokine and stem cell trafficking regulator SDF-1α. On one hand, DPP-4 activity is required for the mobilizing effect of granulocyte colony-stimulating factor (G-CSF), the most commonly used agent to stimulate the egress of stem and progenitor cells toward the bloodstream (124). Therefore, although DPP4-I is not expected to affect G-CSF–induced mobilization, data obtained in patients with diabetes indicate that a maladaptive DPP-4 response to G-CSF contributes impaired mobilization of stem and proangiogenic cells (122). In addition, a screening of factors associated with abnormal stem cell trafficking in patients with diabetes and high cardiovascular risk identified excess DPP-4 activity as a candidate negative modulator of mobilization (125). Using the F344/DuCrlCrlj rat model, it was demonstrated that DPP-4 deficiency improves postischemic BM EPC mobilization and improved microvascular density in the ischemic muscle (125). Therefore, although it is still unknown whether DPP-4 inhibition is able to counteract structural BM remodeling induced by diabetes, modulation of the DPP-4/SDF-1α axis can reverse BM dysfunction and improve microvascular health in distant organs. As the BM is emerging as a central housekeeper, able to affect cellular turnover at distant sites, this hitherto overlooked site of DPP-4 action might uncover interesting and unexpected potentials for microvascular protection in diabetes (20).

CONCLUSIONS

The current standards of care significantly reduce but unfortunately do not eliminate the risk of diabetic microangiopathy. This has important implications because, although microangiopathy is rarely the cause of death in diabetic patients, it is one of the most important risk factors for CVD (126). Furthermore, this implies that in the past years, the most commonly used glucose-lowering drugs were unable to effectively decrease plasma glucose in order to avoid the onset and the progression of microvascular disease. Another relevant issue is that many glucose-lowering agents need to be dose adjusted or should not be used in the setting of stage III–IV CKD or in those receiving dialysis (127). Indeed, the safety of DPP4-I has been demonstrated in several trials in patients with different degrees of renal impairment (128–133). Extensive experimental data and preliminary clinical studies indicate that DPP4-I may improve microvascular structure and function (Table 1 and Fig. 1). Whether the effects of DPP4-I are mediated by improved glucose control or by pleiotropic off-target actions of DPP4-I on nonincretin substrates remains unclear. A few hints can help answer this question with available data (Table 2). Preclinical findings obtained in vitro and using animal models of T1D (e.g., STZ-induced diabetes) suggest that favorable effects of DPP4-I are conveyed independently of glycemic effects. Moreover, short-term studies in T2D patients, showing raised EPCs after just 4 weeks of sitagliptin treatment, are likely exploring pleiotropic rather than glycemic effects. Finally, while lowering HbA_{1c} significantly prevents microangiopathy, the reduced development and progression of microalbuminuria in the SAVOR trial is unlikely to be fully explained by the marginal

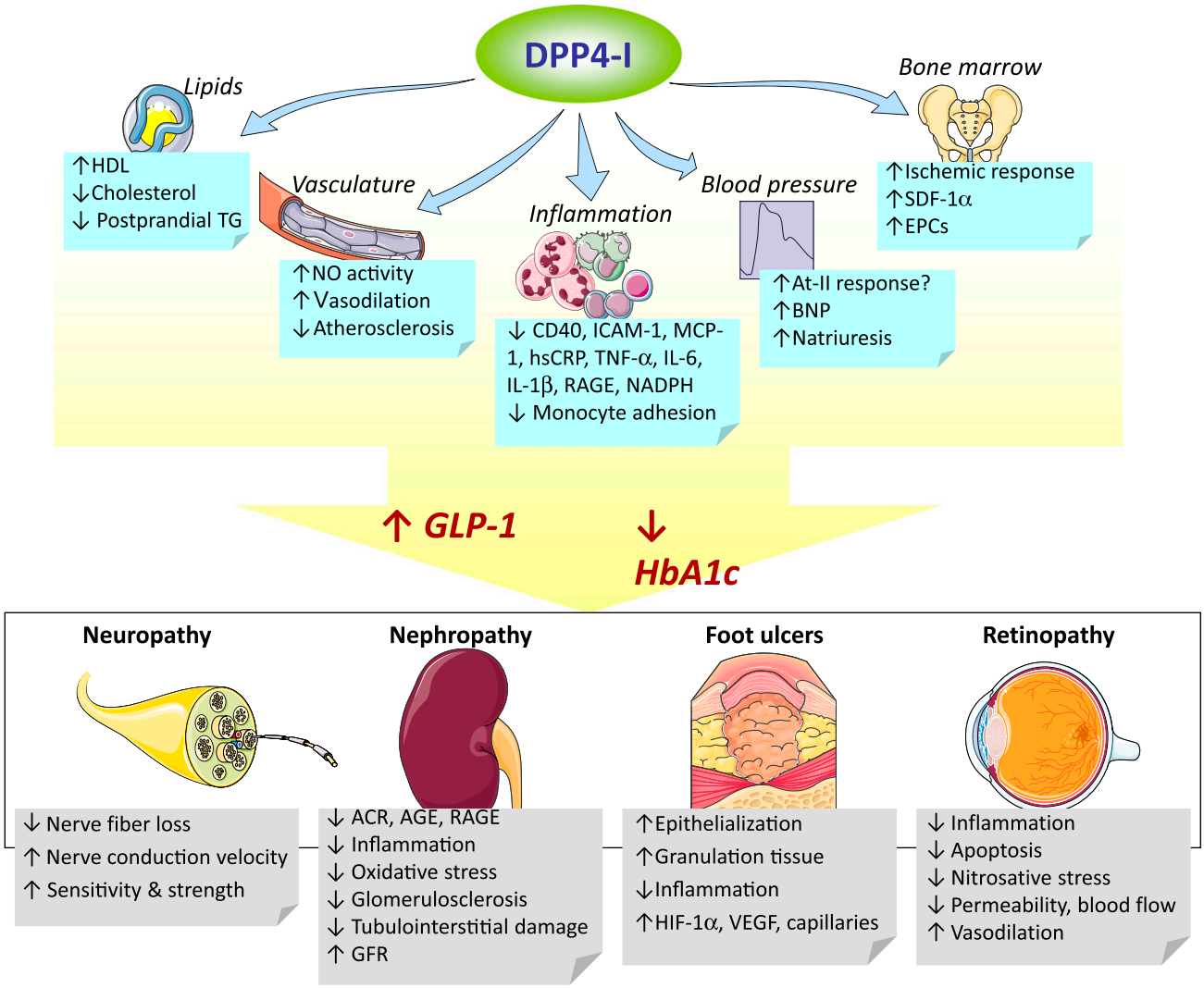

Figure 1—A schematic representation summarizing the roles of DPP-4 inhibition on diabetic microangiopathy. Experimental evidence indicates that DPP4-I affects inflammation, vascular responses, lipids, blood pressure, and BM function. In combination with increased levels of GLP-1 and improved glucose control, these effects can mediate protection from microvascular diabetes complications. BNP, B-type natriuretic factor; GFR, glomerular filtration rate; HIF, hypoxia inducible factor; NO, nitric oxide; TG, triglycerides; VEGF, vascular endothelial growth factor.

0.2–0.3% reduction in HbA_{1c} obtained with saxagliptin compared with placebo throughout the trial (23). However, it must be made clear that preliminary data demonstrate that GLP-1RA have stronger efficacy in terms of correction of the major risk factors for CVD (including blood pressure and lipids) (135); indeed SAVOR, EXAMINE, and other smaller studies did not show any significant effect on both blood pressure and lipids. If these purported protective effects of DPP4-I translate into better outcomes in people with diabetes, they should be verified by the several ongoing clinical trials. Indeed, caution should be paid when trying to translate findings obtained in animal models and small clinical studies to the heterogeneous population of diabetic patients, as long as results from specifically designed randomized controlled trials are not available. In addition to the aforementioned aspects, the effect of DPP4-I on BM stem cells is also promising to achieve microvascular protection at distant sites. Ultimately, reducing the burden of microangiopathy may translate into

Table 2—Potential methodological evidence that the effects of DPP4-I on microvascular protection may be conveyed independently of glucose control

Mechanisms	References
DPP-4 inhibition improves microvascular end points in vitro	(41,134)
DPP-4 inhibition improves microvascular end points in animal models of T1D	(90,92,107–109)
Short-term (4–6 weeks) DPP-4 inhibition in T2D has provided effects that occur before full development of the antihyperglycemic effect	(44,106)
Prevention of microvascular end point is not fully explained by improved glucose control	(23)

improved cardiovascular outcomes in diabetes.

Duality of Interest. A.A. received speaker honoraria from Sanofi, Boehringer Ingelheim, Eli Lilly, Merck Sharp & Dohme, Novartis, Novo Nordisk, Servier, Recordati, Bristol-Myers Squibb, and AstraZeneca and is an advisory member for Boehringer Ingelheim, Novartis, Bristol-Myers Squibb, AstraZeneca, and Sanofi. G.P.F. received speaker honoraria from Eli Lilly, Sanofi, Novo Nordisk, AstraZeneca, and Bristol-Myers Squibb. No other potential conflicts of interest relevant to this article were reported.
Author Contributions. A.A. researched data and wrote, reviewed, and edited the manuscript. G.P.F. researched data and wrote the manuscript.

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