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# Therapeutic potential of dipeptidyl peptidase IV inhibitors for the treatment of type 2 diabetes

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Incretins are peptide hormones, exemplified by glucose-dependent insulinotropic peptide and glucagon-like peptide 1 that are released from the gut in response to nutrient ingestion and enhance glucose-stimulated insulin secretion. Incretin action is terminated due to N-terminal cleavage of the peptides by the aminopeptidase dipeptidyl peptidase IV (DPP-IV). Hence, inhibition of glucose-dependent insulinotropic peptide and glucagon-like peptide 1 degradation via reduction of DPP-IV activity represents an innovative strategy for enhancing incretin action *in vivo*. This review summarises the biology of incretin action, the structure, expression and pleiotropic biological activities of DPP-IV and provides an overview of the rationale, potential merits and theoretical pitfalls in the development of DPP-IV inhibitors for the treatment of type 2 diabetes.

**Keywords:** diabetes, drugs, enzyme inhibitors, GIP, GLP-1, glucagon-like peptides, glucose, incretin, inhibitor, peptidase, peptide

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## 1. Introduction

Incretins are gut peptides, predominantly glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1), which are released from the gastrointestinal tract in response to nutrient ingestion and promote nutrient assimilation via potentiation of glucose-dependent insulin secretion. Incretins, particularly GLP-1, also function in part by contributing to the neurohormonal signals emanating from the distal gut, the 'ileal brake', which regulate the rate through which nutrients transit along the GI tract. The available evidence suggests that enhancement of incretin action may be useful for lowering blood glucose in subjects with type 2 diabetes mellitus. Nevertheless, subjects with type 2 diabetes or obesity may exhibit a diminution in the secretion of endogenous incretins, particularly GLP-1, following food ingestion [1,2]. Furthermore, incretin action is rapidly terminated via the action of dipeptidyl peptidase IV (DPP-IV), which inactivates both GIP and GLP-1 via cleavage at the position 2 alanine. This review provides an overview of incretin and DPP-IV biology, with a focus on critical evaluation of the issues surrounding the use of DPP-IV inhibitors for the treatment of type 2 diabetes.

### 1.1 Glucose-dependent insulinotropic peptide actions

GIP is a 42 amino acid peptide originally characterised as an active component of intestinal extract that inhibited gastric acid secretion, hence its original designation as a gastric inhibitory polypeptide. Subsequent studies demonstrated that GIP exhibited potent insulinotropic properties [3], leading to its concurrent designation as a glucose-dependent insulinotropic polypeptide. GIP directly enhances insulin secretion through a specific GIP receptor expressed on islet β cells [4]. The physio-

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logical importance of GIP action has been delineated in studies using peptide antagonists, GIP receptor knockout mice or antisera directed against the GIP receptor. Acute impairment of GIP action results in defective glucose-stimulated insulin secretion in rats and mice [5,6]. Similarly, mice with targeted disruption of the GIP receptor gene (GIPR-/-) exhibit normal fasting glucose but impaired glucose clearance and reduced insulin secretion following oral glucose challenge [7]. GIP receptors are also expressed on adipocytes, where GIP action may promote fatty acid synthesis and lipid accumulation [8]. Intriguingly, GIPR-/- mice are resistant to weight gain and develop less extensive glucose intolerance following months of high fat feeding, yet exhibit normal feeding behaviour, enhanced fuel oxidation and increased metabolic rate [9]. Furthermore, a double mutant ob:ob/GIPR-/- mouse exhibits less weight gain and relatively improved glucose tolerance compared to the ob:ob mouse alone. These unexpected findings suggest that GIP receptor antagonists merit further analysis in the setting of nutrient-induced obesity and glucose intolerance.

Although GIP stimulates insulin secretion in normal rodents and human subjects, its insulinotropic actions are markedly attenuated in experimental diabetes [10], perhaps due in part to decreased GIP receptor expression and/or function. Similarly, GIP infusion in human subjects with type 2 diabetes does not increase insulin secretion to the same extent seen in normal subjects [11-14], and normoglycaemic relatives of subjects with type 2 diabetes exhibit decreased GIP responsivity [15]. These findings suggest that type 2 diabetes may be associated with both genetic and acquired resistance to GIP action. Hence, there remains comparatively reduced enthusiasm for the use of GIP agonists alone in the treatment of type 2 diabetes, although recent reports suggest that modified protease-resistant GIP receptor agonists [16] may exert glucose-lowering effects in subjects with type 2 diabetes.

### 1.2 Glucagon-like peptide 1 actions *in vivo*

Two equipotent bioactive forms of GLP-1, GLP-1(7-36)amide and GLP-1(7-37), are liberated from proglucagon in enteroendocrine L cells and secreted in response to nutrient intake. Meal-stimulated GLP-1 secretion appears attenuated in human subjects with type 2 diabetes [1,17]. GLP-1 levels fall rapidly following postprandial excursion, and clearance reflects the actions of the kidney [18,19], enzymatic inactivation by DPP-IV [20-22] and, to a lesser extent, neutral endopeptidase (NEP) 24.11 [23]. GLP-1 actions on the islet β cell include stimulation of glucose-dependent insulin secretion [24-26] and induction of glucose competence [27]. GLP-1 also increases insulin gene promoter activity and insulin biosynthesis in cell lines [28,29] and in rodents [30]. GLP-1 also lowers blood glucose via inhibition of gastric emptying [31], thereby attenuating the rate of nutrient entry into the circulation [32], and inhibition of glucagon secretion from islet α cells, probably via effects on insulin or somatostatin secretion [33].

GLP-1 directly stimulates glucose-dependent insulin secretion via an increase in β-cell cAMP [24], through both protein kinase A-dependent and independent mechanisms, with activation of signalling through small G proteins contributing to control of insulin exocytosis [34]. GLP-1 receptor (GLP-1R) activation also promotes calcium mobilisation [35] and closure of the ATP-sensitive $K_{ATP}$ channel [36]. Furthermore, genetic disruption of SUR channel activity in mice is associated with resistance to the insulinotropic actions of both GLP-1 and GIP.

GLP-1 administration to normal or diabetic rodents induces β-cell proliferation [37] and islet neogenesis, leading to an increase in β-cell mass [38,39]. Furthermore, treatment of pancreatic exocrine cell lines with GLP-1R agonists induces a programme of endocrine differentiation associated with molecular features of functional β cells capable of glucose-stimulated insulin secretion [40,41]. The cellular signals coupling GLP-1R activation to islet cell growth appear to include activation of the mitogen-activated protein kinase (MAPK) pathway, protein kinase C and the transcription factor PDX-1 [42]. These observations raise the possibility that GLP-1 may be able to preserve or restore deteriorating β-cell function in type 2 diabetics in part via islet regeneration and augmentation of functional β-cell mass.

GLP-1 also stimulates secretion of hypothalamic-pituitary hormones [43-45] and induces potent anorexic effects following intracerebroventricular administration in rats and mice [46,47]. Furthermore, chronic administration of GLP-1 analogues is associated with weight loss in experimental rodent and primate models of diabetes [48,49], and peripheral GLP-1 administration induces satiety and reduces meal consumption in normal, obese and diabetic human subjects [50,51].

### 1.3 Essential physiological actions of glucagon-like peptide 1

Experiments using GLP-1R antagonists and characterisation of GLP-1R null (GLP-1R-/-) mice have revealed essential physiological actions dependent on GLP-1R signalling. Acute administration of the GLP-1R antagonist exendin(9-39) increases fasting glycaemia and impairs glucose clearance following glucose loading, in association with decreased levels of circulating insulin, in both rodents and human subjects [5,52-55]. Similarly, GLP-1R-/- mice exhibit mild fasting hyperglycaemia and impaired glucose clearance following either oral or intraperitoneal glucose loading [56]. Although chronic intracerebroventricular administration of exendin (9-39) increased food intake and weight gain in rats [57], GLP-1R-/- mice in the CD1 genetic background are lean and do not develop obesity even following prolonged high fat feeding [58]. Moreover, GLP-1R-/- mice exhibit only modest defects in islet size and topography [59] and develop appropriate islet hyperplasia and upregulation of insulin gene expression in response to obesity and insulin resistance [60]. Hence, genetic loss of GLP-1R signalling in the mouse does not produce major perturbations in β-cell growth or insulin biosyn-

thesis, perhaps due to upregulation of compensatory mechanisms, such as enhanced secretion of and sensitivity to GIP [61].

### 1.4 Glucagon-like peptide 1 actions in normal and diabetic human subjects

Consistent with preclinical findings, short-term GLP-1 infusion in normal subjects potentiates glucose-dependent insulin secretion, inhibits glucagon secretion and gastric emptying and produces short-term satiety leading to reduction in food intake [13,50,62,63]. GLP-1 administration to subjects with type 2 diabetes also lowers glycaemia following subcutaneous or intravenous short-term administration [64-67]. GLP-1 also enhances β-cell responsivity to sulfonylurea agents in subjects with type 2 diabetes [68]. The importance of gastric emptying and glucagon secretion for GLP-1 action is exemplified by studies demonstrating glucose-lowering effects of GLP-1 in patients with type 1 diabetes mellitus [62,69].

Native GLP-1 is rapidly degraded to inactive GLP-1(9-37) or GLP-1(9-36)amide [21,22,70]. The plasma half-lifes of GLP-1 (7-36) amide and GLP-1(7-37) as assessed by exogenous infusion of the peptides in human subjects are similar, 5.3 ± 0.4 versus 6.1 ± 0.8 min, respectively, and the metabolic clearance rates of the two biologically active GLP-1 molecules were also comparable (14.6 ± 2.4 versus 12.2 ± 1 pmol/kg x min) [71]. The short duration of endogenous GLP-1 action, taken together with subsequent data demonstrating that a continuous 24-h infusion of GLP-1 was superior to a similar but shorter 16-h infusion for lowering of blood glucose in poorly controlled diabetic subjects [72], provides a sound rationale for development of longer-acting degradation-resistant GLP-1 analogues or continuous infusion approaches for the treatment of patients with type 2 diabetes [73].

### 1.5 Treatment of type 2 diabetes with glucagon-like peptide 1 receptor agonists

Following observations that short-term 24 – 48 h GLP-1 infusions lowered blood glucose in diabetic subjects [74,75], several studies examined the metabolic consequences of longer periods of GLP-1 infusion using the native peptide. A 3-week infusion of GLP-1 in six subjects with type 2 diabetes lowered meal-related glycaemic excursion, increased plasma insulin and decreased plasma glucagon in the postprandial period, with no evidence for tachyphylaxis at the end of the 3-week treatment period [76]. A longer 6-week continuous subcutaneous GLP-1 infusion study in 20 subjects with type 2 diabetes demonstrated significant improvements in mean plasma glucose, fructosamine, haemoglobin (Hb)A1c, fasting and postprandial free fatty acids, with reduced gastric emptying, weight gain and improved β-cell function in the GLP-1-treated subjects [51]. Hence, native GLP-1, if chronically delivered via a continuous infusion strategy, appears highly effective for the treatment of type 2 diabetes.

To circumvent the need for continuous GLP-1 administration, considerable effort has been directed towards the generation of long-acting degradation-resistant GLP-1 analogues suitable for once- or twice-daily administration. Several GLP-1R agonists are currently in clinical trials including the lizard peptide exendin-4 [77] and a fatty acid derivatised DPP-IV-resistant analogue, NN2211 [49]. Intravenous infusion of exendin-4 in normal [63] and diabetic subjects [78] lowers fasting and postprandial plasma glucose [63], stimulates insulin secretion, reduces levels of circulating glucagon and inhibits gastric emptying and food intake. Similarly, subcutaneous administration of NN2211 results in a plasma drug half-life of ~ 12.6 h [79] and lowers both fasting and postprandial glycaemia via effects on insulin and glucagon secretion and gastric emptying [80].

## 2. Dipeptidyl peptidase IV

### 2.1 Structure and expression of DPP IV

DPP-IV, also known as the lymphocyte cell surface protein CD26, is a widely expressed glycoprotein that exhibits three principal biological activities: in humans, it functions as an adenosine deaminase (ADA)-binding protein; it contributes to extracellular matrix binding; and of direct relevance to this review, it exhibits post proline or alanine peptidase activity, thereby inactivating or in some cases generating biologically active peptides via cleavage at the N-terminal region after X-proline or X-alanine (**Box 1**) [81,82]. DPP-IV exists as a membrane bound 110 kDa glycoprotein that is catalytically active as a dimer, whose structure is reasonably well conserved across different mammalian species. The human DPP-IV gene contains 26 exons, is localised to the long arm of chromosome 2 and intriguingly, is localised adjacent to the proglucagon gene which encodes GLP-1 and GLP-2, principal substrates for DPP-IV. The human DPP-IV cDNA encodes a predicted protein of 766 amino acids, with 6 amino acids in the cytoplasm, 22 residues spanning the plasma membrane and 738 amino acids comprising the extracellular domain. DPP-IV also exists as a soluble circulating form of ~ 100 kDa, which retains both adenosine deaminase binding and enzymatic activity and the N-terminal amino acid of the soluble form appears to be Ser39 [83,84]. Consistent with the classical serine protease consensus motif of G-X-S-X-G, the corresponding sequence in DPP-IV is G-W-S-Y-G, with selected mutations in a novel catalytic triad of Ser624, Asp702 and His734 abolishing catalytic activity of the murine molecule [85].

DPP-IV is a widely expressed enzyme present on cells in most tissues, including the kidney, gastrointestinal tract, biliary tract and liver, placenta, uterus, prostate, skin and, of potential relevance to the clinical use of inhibitors, lymphocytes (immune function) and endothelial cells (inactivation of circulating peptides) [86-88]. Furthermore, the expression of DPP-IV in specific tissues or as a circulating soluble form, is widely modulated in the setting of specific diseases or tissue injury and inflammation, as reviewed in [81,89-92] and summarised in **Table 1**.

**Box 1. Putative substrates for dipeptidyl peptidase IV.**

Xaa-Pro
- Tyr-Melanostatin
- Endomorphin-2
- Enterostatin
- B-Casomorphin
- Trypsinogen pro-peptide
- Bradykinin
- Substance P
- CLIP
- Gastrin-releasing peptide (GRP)
- Neuropeptide Y (NPY)
- Peptide YY (PYY)
- Aprotinin
- RANTES
- Granulocyte chemotactic protein-2 (GCP-2)
- Stromal cell-derived factor 1a (SDF-1a)
- Stromal cell-derived factor 1b (SDF-1b)
- Macrophage-derived chemokine (MDC)
- Monocyte chemoattractant protein 1 (MCP-1)
- Monocyte chemoattractant protein 2 (MCP-2)
- Monocyte chemoattractant protein 3 (MCP-3)
- Eotaxin
- Interferon-inducible protein 10 (IP-10)
- Insulin-like growth factor (IGF-I)
- Procolipase
- Interleukin-2 (IL-2)
- Interleukin-1b (IL-1b)
- α1-Microglobulin
- Prolactin
- Trypsinogen
- Human chorionic gonadotrophin (HCG)

Xaa-Ala
- Peptide histidine-methionine (PHM)
- Glucose-dependent insulinotropic peptide (GIP)
- Growth hormone-releasing hormone (GRH)
- Glucagon-like peptide 1 (GLP-1)
- Glucagon-like peptide 2 (GLP-2)

**Box 2. Experimental diseases or conditions modified by DPP-IV inhibition.**

Diabetes
Experimental encephalomyelitis
Murine abortion
Sensitivity to chemotherapy
Invasion, growth and migration of cancer cells
Keratinocyte DNA synthesis
Experimental nephritis
Experimental arthritis

DDP-IV: Dipeptidyl peptidase IV.

### 2.2 CD26/DPP-IV and normal immune function

Originally identified as a lymphocyte cell surface ADA-binding protein with costimulatory activity, CD26 expression and activity are increased following T-cell activation and distinct subpopulations of $CD26^{bright}$ T cells have been identified that subserve multiple functions, including antigen recall, immunoglobulin synthesis and activation of cytotoxic T cells [82]. CD26 associates with other lymphocyte cell surface molecules, including the chemokine receptor CXCR4, ADA and CD45 [89,93], and mAbs against CD26 promote aggregation of both CD26 and CD45 into lymphocyte lipid rafts. Furthermore, CD26 directly binds to the cytoplasmic domain of CD45, providing a mechanism for engagement of specific signal transduction pathways leading to IL-2 production [94], a common downstream event secondary to CD26 activation. Conversely, interleukin induces CD26 expression on a subset of human natural killer lymphocytes [95]. Activation of lymphocyte CD26 leads to increases in intracellular calcium, tyrosine phosphorylation of multiple substrates and cell proliferation [96,97]. CD26 undergoes mannose-6 phosphorylation leading to interaction with the mannose-6-phosphate/insulin-like growth Factor II receptor (M6P/IGFII) receptor following T cell activation [98]. Soluble CD26 also interacts with the (M6P/IGFII) and enhances transendothelial T-cell migration, an effect that requires its DPP-IV enzymatic activity [99].

The majority of experiments assessing lymphocyte CD26 activity use specific antibodies for CD26 activation; whether the enzymatic peptidase activity of CD26 is involved in or required for multiple aspects of lymphocyte signalling has not always been conclusively determined [93]. Experiments carried out with mutant soluble CD26 molecules have demonstrated the importance of DPP-IV enzymatic activity for enhancement of T-cell proliferation and induction of monocyte CD86 expression [100]. Similarly, antiCD26 mAbs inhibit T-cell growth and proliferation via induction of G1/S arrest, effects which are dependent on the enzymatic function of CD26 [101]. Interpretation of data obtained from experiments using specific DPP-IV inhibitors to examine lymphocyte function is complicated by the specificity of the inhibitor employed. However, DPP-IV inhibition has been shown to modify T- and B-cell proliferation and cytokine production, as reviewed in [82]. In contrast, analyses of cells from the CD26/DPP-IV mutant Fischer 344 rat or the CD26/DPP-IV knockout mouse have not yet revealed major defects in lymphocyte activation or immune function [102,103]. The available evidence suggests that the enzymatic activity of DPP-IV may not be essential for many of the T-cell activating or costimulatory properties attributed to CD26. However, not all experiments have used both wild-type and mutant CD26 molecules to examine this specific question.

### 2.3 CD26/DPP-IV activity and disease

DPP-IV activity is increased in patients with cholestatic hepatobiliary disease [104], hepatitis-C-associated liver injury [105] or osteoporosis [106], and in T cells from patients with multiple sclerosis [107] (**Table 1**). CD26 expression and activity may

**Table 1. Human diseases characterised by changes in DPP-IV activity.**

| Human DPP-IV activity | |
|---|---|
| **Increased** | **Decreased** |
| Rheumatoid arthritis | AIDS |
| Multiple sclerosis | Down's syndrome |
| Graves' disease | Common variable |
| Hashimoto's thyroiditis | hypogammaglobulinemia |
| Sarcoidosis | Vasculitis/systemic lupus erythematosis/ |
| Psychological stress | rheumatoid arthritis |
| Cancer | Cancer |
| | Anorexia/bulimia |
| | Depression |
| | Pregnancy |

DPP-IV: Dipeptidyl peptidase IV.

be reduced in T-cell subsets from patients with active HIV [108,109], but increased in HIV-infected subjects with immune reconstitution [110].

In contrast, serum DPP-IV activity is decreased during pregnancy [111], in subjects with active Crohn's disease [112], major depressive illness [113,114], eating disorders [115], active systemic lupus erythematosis [116] or rheumatoid arthritis [117]. Similarly, serum DPP-IV activity is decreased in subjects with active Wegener's granulomatosis, Churg-Strauss syndrome and microscopic polyangiitis, with levels increasing in patients with disease remission [118]. Of potential clinical relevance to diabetes therapeutics, DPP-IV activity is significantly reduced in hypertensive patients treated with angiotensin-converting enzyme (ACE) inhibitors when measured during an episode of drug-associated angioedema [119].

Altered CD26/DPP-IV expression has also been associated with specific cancers, including well differentiated thyroid cancer [120,121] and prostate cancer [122]. Levels are also altered in some patients with colon cancer [123] and oral cancer [124].

### 2.4 DPP-IV enzymatic activity and physiological peptide substrates

A large number of potential peptide substrates for DPP-IV have now been identified, as summarised in **Box 1** and reviewed in [81]. For many of these substrates, evidence implicating a role for DPP-IV in peptide cleavage derives from pharmacological kinetic studies demonstrating that incubation of the peptide and purified enzyme *in vitro* produces peptide cleavage at the N-terminus [125,126]. Whether this line of pharmacological evidence necessarily implies a physiological role for DPP-IV as an essential regulator of peptide activity *in vivo* remains unclear [127]. For example, incubation of 29 amino acid glucagon with purified DPP-IV yields glucagon(3-29) and glucagon(5-29) [128,129] and immunoreactive DPP-IV has been colocalised with glucagon in islet A cell granules [130]. However, increased levels of intact glucagon have not been demonstrated in CD26-/- mice or Fischer 344 DPP-IV mutant rats or following

**Box 3. Criteria for establishing a physiological role for DPP-IV in substrate cleavage.**

Cleavage of the peptide by purified enzymatically active DPP-IV *in vitro*
Peptide degradation in vitro inhibited by DPP-IV inhibitors
Altered ratio of intact to degraded peptide following acute DPP-IV inhibitor administration to normal animals or humans *in vivo*
Altered ratio of intact to degraded peptide substrate in mice or rats with genetic inactivation of DPP-IV

DPP-IV: Dipeptidyl peptidase IV

administration of DPP-IV inhibitors to normal rodents or humans and blood glucose is uniformly lower following administration of DPP-IV inhibitors *in vivo*. Hence, establishment of criteria, as suggested in **Box 3** requiring demonstration that levels of non-cleaved putative DPP-IV substrates are increased in genetic models of DPP-IV deficiency and following administration of DPP-IV inhibitors provides a more rigorous definition for establishing whether specific peptides are physiological (as opposed to pharmacological) targets of DPP-IV enzymatic activity.

The principal known peptide substrates considered major targets of DPP-IV inhibitors when used for the treatment of diabetes are GLP-1 and GIP. Following pharmacological demonstration that purified DPP-IV cleaves both these peptides at the position 2 alanine [20,21], infusion of radiolabelled GIP and GLP-1 into DPP-IV deficient rats revealed almost complete absence of the predicted degradation products, GIP(3-42) and GLP-1(9-36)$NH_2$. Concomitant experiments demonstrated that GIP(3-42) and GLP-1(9-36)$NH_2$ represented the principal degradation products present in human plasma in both the fasting and postprandial states [22]. The degradation of intact GLP-1 occurs rapidly, as GLP-1(9-36)$NH_2$ represents > 50% of detectable immunoreactive GLP-1 released from the isolated perfused porcine ileum [131], with the proportion of intact to N-terminal cleaved GLP-1 greatly increased following administration of DPP-IV inhibitors [131]. Similarly, studies employing structurally unique DPP-IV inhibitors confirmed that increased circulating levels of intact GLP-1 and GIP were detectable following inhibitor administration [132-135]. Furthermore, the proportion of intact to N-terminally degraded GLP-1 and GIP is increased in mice [103] and rats [136] with inactivating mutations of the DPP-IV gene. Hence, both GLP-1 and GIP satisfy multiple criteria (**Box 3**) for designation as physiological peptide substrates of DPP-IV *in vivo*. Although GLP-1(9-36)$NH_2$ is a weak pharmacological antagonist at the GLP-1R, it does not seem to function as a physiologically relevant antagonist *in vivo* [137].

### 2.5 DPP-IV inhibitors and experimental disease

Given the pleiotropic activities of and potential substrates for DPP-IV, the effect of activating and more commonly inhibit-

ing DPP-IV activity has been examined in different experimental models, including neoplastic cell growth. CD26 binds to extracellular matrix components including collagen and fibronectin, potentially modifying cell adhesion, migration and metastatic behaviour. The potential relationship between CD26 expression or activity in neoplastic cells and clinical behaviour of specific tumours, is complex and highly tumour cell-specific. Human T-cell leukaemia Jurkat cells transfected with wild-type DPP-IV or mutant DPP-IV devoid of ADA binding yet retaining enzymatic activity, exhibit increased sensitivity to the cytotoxic effects of doxorubicin [138]. Similarly, soluble CD26 enhanced the growth inhibitory effects of doxorubicin *in vitro*. Consistent with the loss of DPP-IV expression during melanoma progression, inducible re-expression of DPP-IV led to loss of tumorigenicity in human melanoma cells, findings dependent on serine protease activity [139], whereas DPP-IV-transfected melanoma cells exhibited normal growth but reduced migration independent of the proteolytic activity of the enzyme [140]. DPP-IV expression in human ovarian cancer cell lines also correlates with reduced migration, invasion and decreased peritoneal dissemination in nude mice *in vivo* [141]. In contrast, inhibition of DPP-IV activity with diprotin A enhanced invasion of placental JEG-3 cells *in vitro* [142]. Paradoxically, the related membrane bound protease seprase or fibroblast-activating protein, promotes tumour growth and together with DPP-IV, forms a complex on the cell surface that participates in gelatin binding and degradation in migratory fibroblast cells *in vitro* [143]. Hence, the effects of DPP-IV expression and activity on cell growth, migration, invasion and tumorigenicity appear cell- and context-specific.

The importance of DPP-IV expression and activity has also been examined in experimental inflammatory disorders. Treatment of mice with the reversible DPP-IV inhibitor Lys[Z(NO[2])]-pyrrolidide decreased the extent and onset of adoptive transfer experimental autoimmune encephalomyelitis, effects mediated in part through upregulation of transforming growth factor (TGF)-β1 activity [144]. Similarly, DPP-IV inhibitors attenuated the extent of collagen- and alkyldiamine-induced arthritis in rats, and a mAb directed against CD26 suppressed experimental nephritis in rats in association with markedly reduced complement activation [145]. Local DPP-IV expression has been proposed as a modulator of substance-P-induced vasodilatation in the setting of chronic rhinosinusitis [146]. However, the importance of local versus systemic DPP-IV enzymatic activity for the development of inflammation-associated vasodilatation remains uncertain.

### 2.6 DPP-IV-related proteases and specificity of DPP-IV inhibitors

The term DPP-IV activity- and/or structure-homologues has been applied to describe the family of often structurally-related enzymes that exhibit overlapping enzymatic activity with DPP-IV [147]. Several recent reviews have summarised the features of mammalian endo- and exopeptidases capable of cleaving peptides at the N-terminal position 2 alanine or proline (**Box 1**) [81,147]. Hence, rigorous experimental proof is required to implicate an essential physiological role for a specific peptidase in cleavage of peptide substrates *in vivo*. The putative roles of DPP-IV in lymphocyte signalling, cell growth and migration and the importance of enzymatic activity for the cleavage of regulatory peptides have been evaluated with immunoneutralisation and genetic approaches. For example, the availability of rats or mice with inactivating mutations in the DPP-IV gene provides an opportunity to assess the essential or redundant role(s) of the DPP-IV gene in a broad variety of biological systems. Similarly, the binding of DPP-IV to human adenosine deaminase provides an approach for removal of the DPP-IV molecule from specific fluids or extracts, providing a non-genetic approach for the assessment of the biological importance of DPP-IV [84]. Furthermore, experiments employing mutant DPP-IV molecules in which the enzymatic activity of DPP-IV has been specifically inactivated are particularly useful for understanding the contributions of individual CD26 functional domains in a broad spectrum of CD26 biological activities.

In contrast, the use of 'specific' DPP-IV enzyme inhibitors alone to infer biological activities ascribed to DPP-IV is constrained by the difficulty inherent in validating the precise specificity of individual enzyme inhibitors.

## 3. Dipeptidyl peptidase IV inhibitors

### 3.1 DPP-IV inhibition and experimental models of type 2 diabetes

Considerable evidence from studies in rats, mice, dogs and human subjects attests to the concept and efficacy of using DPP-IV inhibitors for the treatment of diabetes [148], and has recently been reviewed [149,150]. The inhibitor valine pyrrolidide (Val-pyr) reduced porcine plasma DPP-IV activity by > 90% and decreased the degradation of intact GLP-1, both in the fasted state and following exogenous GLP-1 administration [151]. Infusion of glucose together with GLP-1 in the presence of Val-pyr produced a significant augmentation in levels of plasma insulin compared to GLP-1 infusion in the absence of the inhibitor [151]. The DPP-IV inhibitor isoleucine thiazolidide (Ile-thiazolidide) prevented N-terminal degradation of both GLP-1 and GIP in human serum, and oral administration of Ile-thiazolidide to both lean or obese Zucker fatty rats inhibited plasma DPP-IV activity, decreased glycaemic excursion and enhanced levels of circulating insulin following oral glucose loading [152]. In contrast, administration of the inhibitor alone without concomitant glucose loading had no effect on levels of fasting glucose or insulin in obese Zucker rats [152].

Analysis of rat plasma following administration of both Ile-thiazolidide and radiolabelled GLP-1 demonstrated that 70% inhibition of rat plasma DPP-IV activity markedly reduced the degradation of exogenous [125I]-labelled GLP-1(7-

36)$NH_2$ [132]. Furthermore Ile-thiazolidide reduced glycaemic excursion, enhanced levels of plasma insulin and prolonged the half-life of endogenous GLP-1(7-36)$NH_2$ released following intraduodenal glucose loading [132]. Similar results were obtained following administration of the inhibitor NVP-DPP728 to lean and obese Zucker rats, with enhanced insulin release and reduced glycaemic excursion detected in the inhibitor-treated rats, in association with markedly enhanced levels of intact GLP-1(7-36)$NH_2$ [133].

The glucose-lowering properties of Val-Pyr were subsequently examined in C57BL/6 mice following 5 weeks of high fat (58% total fat) feeding. Consistent with previous findings, Val-Pyr markedly augmented the plasma levels of GLP-1 following intravenous GLP-1 administration to normal C57BL/6 mice, and acute inhibitor administration decreased glycaemic excursion and increased levels of both insulin and GLP-1 following oral glucose loading in both high fat fed and control mice [153]. In contrast, Val-Pyr had no effect on glucose-stimulated insulin secretion from isolated islets *in vitro*. The importance of GIP as a substrate for DPP-IV inhibitors is illustrated by experiments in mice and pigs. Administration of Val-Pyr to GLP-1R knockout mice produces a glucose-lowering effect, suggesting that DPP-IV substrates independent of GLP-1 are also important for glucose clearance *in vivo* [103]. Similarly, Val-Pyr markedly reduces the N-terminal degradation of intact GIP and potentiates the insulinotropic actions of infused GIP in pigs [154].

More recent studies have examined the effects of chronic DPP-IV inhibitor administration in rodent models of type 2 diabetes. Oral administration of P32/98, 20 mg/kg b.i.d. for 3 months was associated with a progressive improvement in fasting glucose over the 12-week study period, in association with enhanced levels of glucose-stimulated insulin, a 12.5% decrease in relative body weight gain and improvements in insulin sensitivity as assessed at the end of the treatment period [155,156]. Inhibitor-treated rats exhibited enhanced insulin release following pancreatic perfusion with 8.8 mM glucose, increased insulin-stimulated adipose tissue glycogen synthase activity and increased insulin-stimulated methyl glucose uptake in soleus muscle strips [155]. The mechanism by which incretin hormones increase insulin sensitivity remains unclear, however, similar findings have been observed in human diabetic subjects treated with continuous GLP-1 infusion for 6 weeks [51]. Interestingly, despite the marked improvements in glucose homeostasis observed in inhibitor-treated rats, fasting levels of plasma DPP-IV activity were significantly increased in P32/98-treated rats. However, the precise source of increased circulating plasma DPP-IV remains unclear [155].

Chronic inhibition of DPP-IV activity has also been studied in Zucker diabetic fatty rats treated once- or twice-daily with the long-acting inhibitor FE 99901. This compound produced comparatively greater and sustained inhibition of plasma DPP-IV activity compared to similar doses of NVP-DPP728 after single dosing, and a 7-day treatment period with FE 99901 improved glucose tolerance in association with increased levels of glucose-stimulated insulin [157]. Chronic twice-daily treatment with FE 99901 for 25 days significantly delayed the deterioration in plasma glucose observed in control rats treated with vehicle alone, in association with a reduction in food intake and water consumption and modest but significant increases in the levels of circulating GLP-1. Furthermore, FE 99901-treated rats displayed significant reductions in levels of free fatty acids and triglycerides and increased pancreatic expression of the GLP-1R [157]. Twice-daily inhibitor administration was significantly more effective than once-daily treatment, attesting to the importance of sustained suppression of plasma and/or tissue DPP-IV activity for optimal glucose control.

The effect of an 8-week treatment period using NVP-DPP728 was examined in C57BL/6 mice fed a high fat diet [135]. Treatment was commenced at 5 weeks of age and NVP-DPP728 was added continuously in the drinking water at a concentration of 0.12 µmol/g body weight, resulting in marked suppression of plasma DPP-IV activity to < 5% of control values. Inhibitor-treated mice fed normal or high fat diets did not exhibit differences in body weight, but cumulative food intake was significantly reduced in high fat fed mice treated with NVP-DPP728 when assessed during the last week of the study period [135]. Glucose tolerance improved and both circulating insulin and GLP-1 levels increased following 8 weeks of inhibitor treatment in normal or high fat fed mice [135]. Furthermore, glucose-stimulated insulin secretion was improved in isolated islets from inhibitor-treated mice and islet size was smaller in mice treated with NVP-DPP728 [135]. Hence the available evidence from a variety of rodent models supports the efficacy of chronic DPP-IV inhibitor administration for the treatment of experimental type 2 diabetes.

### 3.2 DPP-IV inhibition and the treatment of human subjects with type 2 diabetes

Only limited information is currently available concerning the clinical efficacy of DPP-IV inhibitors in the treatment of human subjects with type 2 diabetes. NVP-DPP728 has been administered in a placebo-controlled, double-blind, multicentre study either at 100 mg t.i.d or 150 mg orally b.i.d. for 4 weeks to 93 patients with diet-controlled type 2 diabetes; mean age 64, prior duration of diabetes ~ 3.6 years, body-mass index (BMI) 27.2, with a mean fasting glucose of 8.5 and a HbA1c of 7.4% prior to drug treatment [158]. Both treatment regimens significantly improved mean 24-h glucose excursion with a reduction in mean 24-h insulin levels noted in treated subjects. Fasting and postprandial plasma glucose was also significantly reduced in both treatment arms, as was HbA1c. Body weight was not changed during the 4 week study period. Four drug-treated patients experienced symptoms compatible with nasopharyngitis and five patients complained of pruritus primarily localised to the palms. However, these symptoms were transient, with pruritus disappearing within 48 h without need for discontinuation of therapy. One patient with pre-existing albuminuria developed transient

nephrotic syndrome during the first week of treatment, leading to discontinuation of therapy.

The efficacy of the orally available inhibitor P32/98 has also been examined in both healthy normal subjects and in patients with type 2 diabetes [159]. A single 60 mg oral dose produced a rapid inhibition of plasma DPP-IV activity within 45 min of drug administration. When P32/98 was given 15 min prior to an oral glucose tolerance test in healthy volunteers, increased levels of bioactive intact GLP-1 were detected in drug-treated subjects. Analysis of the effects of single dose P32/98 on glucose excursion in diabetic subjects revealed reduced glucose area under the curve for patients previously treated with acarbose and glibenclamide [159]. The effects of long-term treatment with P32/98 in diabetic subjects have not yet been reported.

**Table 2. Comparison of DPP-IV inhibitors versus GLP-1 analogues for the treatment of type 2 diabetes.**

| DPP-IV inhibitors | GLP-1 analogues |
|---|---|
| Orally available | Injectable |
| Multiple targets | Single known GPCR target |
| Stabilisation of endogenous GLP-1 | Higher levels of circulating GLP-1 achievable |
| Short versus long acting | Longer acting; days to weeks? |
| Drug overdose non-toxic | Drug overdose potentially problematic |
| CNS side effects unlikely | Potential for CNS side effects |
| Potential for unanticipated toxicity | Biological actions more precisely defined |

DPP-IV: Dipeptidyl peptidase IV; GLP-1: Glucagon-like peptide 1; GPCR: G-protein-coupled receptor.

## 4. Expert opinion

The important observations that both GLP-1 and GIP are rapidly cleaved at the N-terminus, followed by the identification of DPP-IV as an essential determinant of incretin inactivation, has fostered considerable interest in evaluation of DPP-IV inhibitor therapy for the treatment of type 2 diabetes. Concurrently, multiple long-acting GLP-1R agonists are being evaluated in the clinic in Phase II/III trials in diabetic subjects. The theoretical advantages of GLP-1R agonists (**Table 2**) include the ability to achieve much greater and sustained levels of circulating bioactive GLP-1, which should provide more robust and sustained activation of GLP-1Rs coupled to glucose lowering. Furthermore, injectable GLP-1 analogues are likely to be more potent inducers of satiety and inhibitors of gastric emptying, and they have been shown to regulate islet cell proliferation and cytoprotection. In contrast, although DPP-IV inhibitors are orally available and potentially more attractive to patients, they are less well characterised with respect to their spectrum of incretin-like actions and safety profile (**Table 2**) and are predicted to be less potent than injectable GLP-1 analogues in the acute lowering of plasma glucose.

Hence, several important questions and challenges remain if this class of agents is to be developed successfully and safely for use in the diabetes clinic. The large number of potential DPP-IV substrates, encompassing gut and CNS regulatory peptides, chemokines and vasoactive peptides, suggests that predicting and understanding the biology of transient or sustained DPP-IV inhibition in human subjects may be difficult, even after exhaustive preclinical evaluation of highly specific compounds. Furthermore, the pleiotropic functions of DPP-IV, acting as both a membrane bound and soluble form and exerting diverse effects on lymphocyte signalling, cell migration and proliferation, at times independent of its enzymatic activity, provide further challenges for scientists seeking to understand how specific inhibition of enzymatic function may impact the non-enzymatic biological actions of DPP-IV in different human tissues. The relative long-term safety of compounds that produce transient versus more sustained DPP-IV inhibition cannot be inferred from available data, although studies of incretin biology and preclinical evaluation of DPP-IV inhibitors argue that continuous potentiation of incretin receptor signalling is likely to be more effective for the treatment of subjects with type 2 diabetes mellitus.

Although the pharmaceutical industry is developing multiple potent, highly specific, DPP-IV inhibitor compounds with favourable pharmacokinetic profiles, the biology and consequences of sustained DPP-IV inhibition may be different in comparatively well patients with type 2 diabetes versus more complex older diabetic subjects with additional coexisting illnesses. For example, the effects and putative safety of chronic DPP-IV inhibitor therapy in diabetic patients with coexistent immune or inflammatory disorders, atopy, angioedema or malignancy, cannot be inferred with any degree of confidence from preclinical or short-term clinical studies. Nevertheless, despite these concerns, the surprising potency of these compounds in experimental models of type 2 diabetes, the need for new effective medications to treat type 2 diabetes, taken together with the preliminary data demonstrating efficacy in short-term clinical trials, argues for ongoing assessment and evaluation of these compounds as new therapeutic agents. More selective approaches for targeting the DPP-IV enzyme, for example with tissue-specific inhibitors, are under development and may offer theoretical advantages for restricting drug activity to one or more localised tissue compartments. Similarly, DPP-IV inhibitors have been proposed as agents for the treatment of immune or CNS disorders. However, insufficient data are available to provide informed opinion about the scientific merits of these strategies. As is the case for all new investigational agents representing innovative approaches to disease treatment, there will be no substitute for rigorous scientific assessment of the specificity, mechanisms of action, safety and efficacy for each new DPP-IV compound that enters clinical development for the treatment of type 2 diabetes.

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