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## Inhibition of dipeptidyl peptidase IV activity as a therapy of Type 2 diabetes

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Dipeptidyl peptidase IV (DPP IV) is a ubiquitous, multifunctional, serine protease enzyme and receptor with roles in the control of endocrine and immune function, cell metabolism, growth and adhesion. As an enzyme, DPP IV cleaves the N-terminal dipeptide from the incretin hormones glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide. This inactivates the hormones, thereby cancelling their prandial insulinotropic effect. One approach to restore incretin activity as a therapy for Type 2 diabetes has been the development of DPP IV inhibitors. Inhibitors of DPP IV have shown efficacy and tolerability when used to control the hyperglycaemia of non-insulin-dependent animal models and human Type 2 diabetes. These DPP IV inhibitors prolong active incretin hormone concentrations and may exert additional antidiabetic effects. If long-term clinical trials confirm sustained and safe control of blood glucose, DPP IV inhibitors (known as 'gliptins') may be expected to provide a new treatment modality for Type 2 diabetes.

**Keywords:** diabetes, dipeptidyl peptidase IV, incretin hormones

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### 1. Introduction

The concept of dipeptidyl peptidase IV (DPP IV) inhibitors arose through improvements in our understanding of the physiological inactivation of incretin hormones. The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are sister hormones that potentiate postprandial insulin secretion and glucose clearance [1]. These peptides have pleiotropic effects on a range of tissues. Key actions, shown in Table 1, include the stimulation of insulin release from the pancreas [2], reduction of hepatic insulin clearance [3] and insulin-like effects on skeletal muscle [4-6], liver [7] and adipose tissue [8-11], which serve to promote glucose uptake and metabolism. GLP-1 and GIP have beneficial actions on the pancreatic  $\beta$ -cell, such as expansion of cell mass and increased cell survival [12].

The primary amino acid sequence of GLP-1 and GIP reveals a highly conserved alanine penultimate to the N-terminus, thus making these peptides ideal putative substrates for DPP IV (Figure 1). The major metabolites generated by DPP IV processing of GLP-1 and GIP, namely GLP-1(9-36)amide and GIP(3-42), respectively, retain the ability to bind to their specific receptors, but are rendered non-insulinotropic (Figure 2) [13-16]. The action of ubiquitous DPP IV reduces the half-life *in vivo* of GLP-1 and GIP to < 2 min [13,17,18]. The timing of these findings coincided with other observations that demonstrated the therapeutic potential of incretin hormones in relation to Type 2 diabetes [2]. Therefore, two strategies were conceived to harness the antidiabetic potential of incretin hormones. Initially came the development of analogues of GLP-1 and GIP resistant to DPP IV (see recent reviews [1,19]), and thereafter came the development of inhibitors of DPP IV. It is the

**Table 1. Overview of functional characteristics of GLP-1 and GIP.**

	GLP-1	GIP
Released in response to a mixed meal	√	√
Lower blood glucose	√	√
Glucose-dependent stimulation of insulin secretion	√	√
Suppress glucagon secretion	√	-
Enhance β-cell survival	√	√
Stimulate β-cell expansion	√	√
Extrapancreatic glucose-lowering actions	√	√
Suppress gastric acid secretion	-	√
Inhibition of gastric emptying	√	-
Inhibition of hepatic insulin extraction	√	√
Enhance satiety	√	-
Reduce body weight	√	-

√: Yes; -: Effect uncertain; GIP: Glucose-dependent insulinotropic polypeptide; GLP-1: Glucagon-like peptide-1.

aim of this review to summarise the most recent and important advances in the development of DPP IV inhibitors as a new drug class for the treatment of Type 2 diabetes [20,21]. In particular, how the recent discovery of several DPP IV-related proline specific peptidases has prompted a re-evaluation of DPP IV inhibitors and their degree of selectivity is discussed.

## 2. Background

### 2.1 Type 2 diabetes

Type 2 diabetes represents ~ 90% of all cases of diabetes and is characterised by two main defects: i) impairment of pancreatic β-cell function, and ii) impairment of insulin sensitivity of muscle, adipose tissue and liver. Ideally, future treatment strategies should seek to address both of these defects, as well as the resultant hyperglycaemia [22,23]. Type 2 diabetes is a major debilitating illness throughout the world and resulting complications place a growing burden on healthcare budgets.

### 2.2 DPP IV and related enzymes

Despite the vast array of different proteases found physiologically, few can cleave the peptide bond following a proline amino acid residue. Fewer still can cleave this bond when it is located just two positions from the N-terminus. Serine proteases that carry out this specific cleavage function are termed the 'postproline dipeptidyl aminopeptidases'. Many of these proteases belong to the DPP IV gene family. The family of enzymes related to DPP IV comprise: i) DPP IV, ii) fibroblast activation protein (FAP), iii) DPP 8, iv) DPP 9 and v) DPP II, also known as DPP 7 or quiescent cell proline dipeptidase (QPP) [24]. DPP IV is usually identified by its postproline aminopeptidase activity, that is, its ability to preferentially cleave Xaa-Pro or Xaa-Ala dipeptides from the N-terminus of polypeptides (where Xaa is any amino acid except Pro).

#### 2.2.1 DPP IV (EC 3.4.14.5)

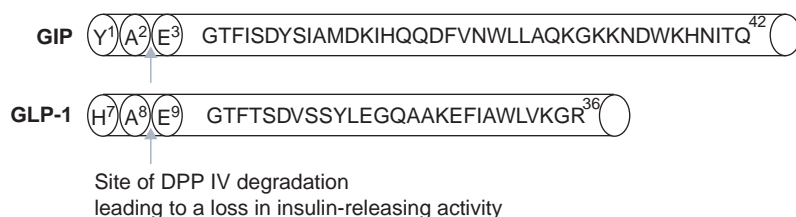
DPP IV doubles as the cell-surface CD26 T-cell-activating antigen and is expressed in almost all organs and tissues [25]. In humans it is strongly expressed in the exocrine pancreas, kidney, gastrointestinal tract, biliary tract, thymus, lymph nodes, uterus, placenta, prostate, adrenal, sweat glands, salivary and mammary glands. It is also found on endothelia of all organs examined, including spleen, lungs, brain and vessels supplying the liver [26]. In addition to being a cell-surface ectoenzyme anchored to the plasma membrane, DPP IV is found solubilised in body fluids such as blood plasma and cerebrospinal fluid. The distribution of DPP IV activity gives it ready access to endocrine peptides, neuropeptides and a wide range of paracrine and autocrine peptides and polypeptides.

#### 2.2.2 FAP

FAP is a type II membrane-bound serine protease with 52% similarity to DPP IV. There has been speculation that FAP could be involved in wound healing as well as tumour growth and proliferation. It has also been linked with liver injury and chronic liver disease [27,28]. FAP is capable of dipeptidyl peptidase activity to cleave N-terminal dipeptides from polypeptides, and collagenolytic activity that can degrade gelatin and type I collagen [29]. A common active site in FAP is used for both functions [29]. Immunopurified recombinant FAP possesses DPP IV-like activity, as demonstrated by its ability to cleave an Ala-Pro-NH F<sub>3</sub> Mec substrate [27]. FAP does not appear to be as ubiquitously expressed as other members of the DPP IV enzyme family, but has been found in serum and the α-cells of the pancreas [28].

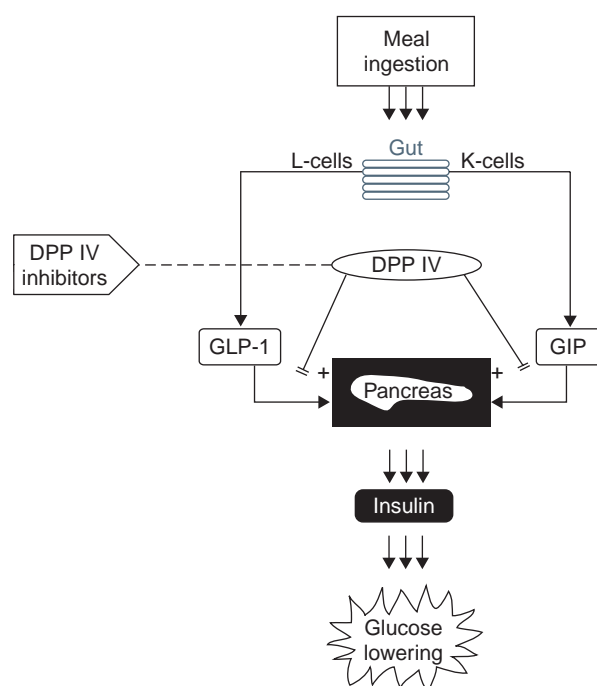
#### 2.2.3 DPP 8 and 9

DPP 8 and 9 are soluble postproline cleaving dipeptidases localised in the cytoplasm. Although DPP 8 and 9 share ~ 50% amino acid similarity to human DPP IV, they appear



**Figure 1. Degradation of incretin hormones by DPP IV.** Incretins GLP-1 and GIP possess a highly conserved alanine amino acid residue penultimate to the N-terminus, making them ideal putative substrates for DPP IV. His<sup>7</sup>-Ala<sup>8</sup> and Tyr<sup>1</sup>-Ala<sup>2</sup> dipeptides are removed from GLP-1 and GIP, respectively, leading to noninsulinotropic metabolites GLP-1(9-36)amide and GIP(3-42).

DPP IV: Dipeptidyl peptidase IV; GIP: Glucose-dependent insulinotropic polypeptide; GLP-1: Glucagon-like peptide-1.



**Figure 2. DPP IV inhibitor mode of action.** The incretin hormones GLP-1 and GIP, released from the L- and K-cells of the intestine, stimulate insulin release from the pancreas, leading to lower plasma glucose concentrations. However, enzymatic cleavage by ubiquitous DPP IV renders them noninsulinotropic. DPP IV inhibitors prevent processing by DPP IV and, therefore, improve incretin-stimulated insulin release and glucose lowering.

DPP IV: Dipeptidyl peptidase IV;  
GIP: Glucose-dependent insulinotropic polypeptide;  
GLP-1: Glucagon-like peptide-1.

to be more closely related to each other (~76% similarity) [30]. They are widely distributed in human tissues, but have not yet been associated with any particular biological process. DPP 8 and 9 are active as monomers and hydrolyse H-Ala-Pro- and H-Gly-Pro-derived substrates [28]. It remains a distinct possibility that many of the functions ascribed to DPP IV may actually be derived from the activity of DPP 8 and/or 9. The

recent discovery of DPP 8 and 9 has had major implications for the specificity of DPP IV inhibitors, to avoid inhibition of DPP 8 and 9.

#### 2.2.4 DPP II (DPP 7 or QPP) (E.C. 3.4.14.2)

DPP II was originally referred to as dipeptidyl aminopeptidase II [31] and its activity was detected by the hydrolysis of Lys-Ala-derived chromogenic or fluorogenic substrates at acidic pH. DPP II activity has been detected in a range of mammalian tissues [32]. Recent evidence has strongly indicated that DPP II, DPP 7 and QPP are not different enzymes, but in fact the same enzyme with three different names in the literature [32,33]. Although only a few compounds have been discovered with an inhibitory effect on DPP II activity, they were all originally described as DPP IV inhibitors. Compounds such as Val-boro-Pro and Ala-Pyrr-2-CN, as well as aminoacyl pyrrolidines and thiazolidine derivatives actually have a higher potency towards DPP IV [34-36]. There are two compounds, Ala-ψ[CS-N]-Pyrr and Ala-ψ[CS-N]-Thia, with more selectivity towards DPP II than DPP IV [37].

#### 2.3 Natural substrates of DPP IV enzyme activity

Table 2 lists examples of the extensive range of physiological regulatory peptides identified as substrates or potential substrates of DPP IV. All of these peptides have either Ala, Pro or Ser penultimate to the N-terminus. It is evident that DPP IV acts on several chemokines that affect the immune system. Chemokine substrates of DPP IV include RANTES, eotaxin, interferon-γ-inducible protein-10, monocyte chemoattractant protein (MCP)-1, -2 and -3, stromal cell-derived factor-1α and -β, granulocyte chemotactic protein-2 and macrophage-derived chemokine (see Table 2) [25]. By altering chemokine activity, DPP IV can modify specificity for, and ability to activate, immune receptors. For example, the peptide RANTES(1-68), which is chemotactic for lymphocytes, monocytes, dendritic cells, eosinophils, basophils and NK cells, is truncated to RANTES(3-68) by DPP IV. RANTES(3-68) is unable to increase cytosolic calcium levels and induce chemotaxis in human monocytes [38]. Furthermore, RANTES(3-68) antagonises the chemotactic effects of RANTES(1-68) and other chemotactic proteins

**Table 2. Physiological regulatory peptides identified as substrates of DPP IV**

Peptide	N-terminus	Reference
GLP-1 (7-36)amide	His-Ala-Glu-	[101]
GLP-1 (7-37)	His-Ala-Glu-	[101]
GLP-2 (1-33)	His-Ala-Asp-	[102]
GIP (1-42)	Tyr-Ala-Asp-	[101]
GHRH	Tyr-Ala-Glu-	[101]
GHRH (1-29)	Tyr-Ala-Asp-	[103]
GHRH (1-44)	Tyr-Ala-Asp-	[101]
Peptide histidine methionine	His-Ala-Asp-	[101]
PACAP (1-27)	His-Ser-Asp-	[44-46]
PACAP (1-38)	His-Ser-Asp-	[44-46]
Gastrin-releasing peptide	Val-Pro-Leu-	[104]
Substance P	Arg-Pro-Lys-	[104]
Insulin-like growth factor-1	Gly-Pro-Glu-	[25]
Bradykinin	Arg-Pro-Pro-	[105]
Neuropeptide Y	Tyr-Pro-Ser-	[106]
Peptide YY (1-36)	Tyr-Pro-Ile-	[106]
Prolactin	Thr-Pro-Val-	[104]
Human chorionic gonadotropin- $\alpha$	Ala-Pro-Asp-	[104]
Luteinising hormone $\alpha$ -chain	Phe-Pro-Asn-	[25]
Thyrotropin- $\alpha$	Phe-Pro-Asp-	[25]
Enkephalins	Tyr-Pro-Val-	[107]
Vasostatin-1	Leu-Pro-Val-	[108]
Trypsinogen	Phe-Pro-Thr-	[104]
Trypsinogen propeptide	Phe-Pro-Thr-	[104]
Procolipase	Val-Pro-Asp-	[104]
IL-2	Ala-Pro-Thr-	[104]
IL-1 $\beta$	Ala-Pro-Val-	[109]
$\alpha_1$ -Microglobulin	Gly-Pro-Val-	[104]
Tyr-melanostatin	Tyr-Pro-Leu-	[104]
Endomorphin-2	Tyr-Pro-Phe-	[110]
Enterostatin	Val-Pro-Asp-	[111]
$\beta$ -Casomorphin	Tyr-Pro-Phe-	[104]
Corticotropin-like intermediate lobe peptide	Arg-Pro-Val-	[104]
Aprotinin	Arg-Pro-Asp-	[104]
RANTES	Ser-Pro-Tyr-	[112]
Granulocyte chemotactic protein-2	Gly-Pro-Val-	[38]
SDF-1 $\alpha$	Lys-Pro-Val-	[113]
SDF-1 $\beta$	Lys-Pro-Val-	[113]
Macrophage-derived chemokine	Gly-Pro-Tyr-	[114]
MCP-1	Glu-Pro-Asp-	[38]
MCP-2	Glu-Pro-Asp-	[112]

DPP IV: Dipeptidyl peptidase IV; GHRH: Growth hormone-releasing hormone; GIP: Glucose-dependent insulinotropic polypeptide; GLP-1: Glucagon-like peptide-1; MCP: Monocyte chemotactic protein; PACAP: Pituitary adenylyl cyclase-activating peptide; SDF: Stromal cell-derived factor.

**Table 2. Physiological regulatory peptides identified as substrates of DPP IV (continued)**

Peptide	N-terminus	Reference
MCP-3	Glu-Pro-Val-	[38]
Eotaxin	Gly-Pro-Ala-	[112]
IFN- $\gamma$ -inducible protein-10	Val-Pro-Leu-	[112]

DPP IV: Dipeptidyl peptidase IV; GHRH: Growth hormone-releasing hormone; GIP: Glucose-dependent insulintropic polypeptide; GLP-1: Glucagon-like peptide-1; MCP: Monocyte chemotactic protein; PACAP: Pituitary adenyl cyclase-activating peptide; SDF: Stromal cell-derived factor.

such as macrophage inflammatory protein-1 $\alpha$  and -1 $\beta$  and MCP-3 [39].

Neuropeptide substrates of DPP IV include substance P, bradykinin, peptide YY (PYY), neuropeptide Y (NPY), endomorphin and pituitary adenyl cyclase activating peptide (PACAP). With their conserved Tyr-Pro N-terminus, PYY and NPY appear to be convenient substrates for DPP IV [25]. NPY normally stimulates food intake and promotes weight gain [40] and this effect could be reduced by DPP IV. The specificity of PYY(1-36) for receptor subtypes is significantly altered following cleavage to PYY(3-36). Whereas PYY(1-36) binds to and activates at least three Y receptor subtypes (Y1, -2 and -5), PYY(3-36) is selective for the Y2 receptor. PYY(3-36) has a satiety effect, acting via the Y2 receptor in the arcuate nucleus to reduce food intake [41]. It remains to be seen whether inhibition of DPP IV activity could change the balance of PYY(1-36) and PYY(3-36), and potentially increase food intake or interfere with effects of NPY and GLP-1 on food intake. PACAP is another neuropeptide substrate of DPP IV. PACAP is a member of the glucagon superfamily of peptides and, therefore, is closely related to the incretin hormones. PACAP is localised in pancreatic islet nerves and islet  $\beta$ -cells [42,43], and like the incretins, PACAP is an insulintropic peptide. PACAP is found in active 1-27 and 1-38 amino acid isoforms, which are degraded to PACAP(3-27) and PACAP(3-38), respectively [44-46]. PACAP(3-27) and PACAP(3-38) fragments are noninsulintropic [44]. It appears that PACAP(3-27) undergoes further degradation to PACAP(5-27) and PACAP(6-27), neither of which are insulintropic. Interestingly, PACAP(6-27) appears to antagonise the actions of PACAP(1-27). Similarly, DPP IV truncates glucagon to generate glucagon(3-29) and glucagon(5-29). This substantially reduces affinity for the glucagon receptor and removes biological activity [47].

#### 2.4 The incretin hormones as substrates of DPP IV

The two major incretin hormones, GLP-1 and GIP, are degraded by DPP IV [1,2,48]. These peptide hormones are 30 and 42 amino acids in length, respectively, and the action of DPP IV removes an N-terminal dipeptide from each. Physiologically, DPP IV degrades GLP-1(7-36)amide and GLP-1(7-37) into major truncated metabolites GLP-1(9-36)amide and GLP-1(9-37) (Figure 1). Similarly, GIP(1-42) is truncated to GIP(3-42) (Figure 1). These truncated peptides are rendered noninsulintropic. However, recent evidence suggests that

truncated GLP-1(9-36)amide may retain other biological functions, particularly in relation to glucose disposal [49]. In healthy male volunteers, GLP-1(9-36)amide lowers postprandial glycaemia independent of any effects on insulin secretion, glucagon secretion or the rate of gastric emptying [50]. Therefore, the action of DPP IV on incretin hormones may be confined to an effect on insulin secretion and not on other glucoregulatory effects of these hormones.

### 3. Medical need

Type 2 diabetes represents a large and growing unmet medical need. In developed countries the prevalence of Type 2 diabetes is rising rapidly, and at least a third of cases are undiagnosed. The resulting comorbidities (e.g., retinopathy, neuropathy, nephropathy) associated with Type 2 diabetes are placing larger burdens on health services and budgets. Several of the current drug therapies have been associated with secondary failure as well as undesirable side effects, such as hypoglycaemia.

### 4. Existing treatment

Although dietary control is always the primary approach to Type 2 diabetes, and tackling obesity will enhance insulin sensitivity and glucose control [22,23], moderate or severe hyperglycaemia invariably requires drug intervention [22,23]. Current therapies improve metabolic abnormalities by either enhancing insulin secretion (sulfonylureas, meglitinides) or reducing insulin resistance (biguanides, thiazolidinediones). All of the available agents are limited in efficacy by the progressive deterioration of  $\beta$ -cell function that occurs throughout the natural history of Type 2 diabetes. Improvements in our understanding of the pancreatic  $\beta$ -cell function are necessary to address current unmet medical needs and achieve better glycaemic control in more patients. Compounds based on the physiological incretin hormones, GLP-1 and GIP, may meet such needs. The first GLP-1 analogue, exenatide, was launched in the US in 2005 and other stable analogues are expected soon. There are indications that incretin hormones may prevent or even reverse the gradual  $\beta$ -cell decline, thus making them a particularly advantageous addition to current treatments. DPP IV inhibitors seek to improve the action of endogenous incretin hormones, thereby representing a potential new addition to the growing armoury of antidiabetic drugs.

Table 3. DPP IV inhibitor compounds in development or recently discontinued.

Inhibitor	Type of action	Company	Status	Phase	Specificity/ inhibitory coefficient
P32/98 (isoleucine thiazolidide)	Reversible product analogue	Probiodrug		II	$K_i = 80$ nM
Sitagliptin (MK-0431)	Reversible product analogue	Merck	NDA submitted to FDA 2006	III	$IC_{50} = 18$ nM
Vildagliptin (LAF-237)	Reversible product analogue	Novartis	NDA submitted to FDA 2006	III	$IC_{50} = 3.5$ nM
NVP-DPP728	Covalently bound product analogue	Novartis	Discontinued	II	$IC_{50} = 22$ nM
Aminomethylpyridine (R-1438)	Reversible nonpeptide heterocyclic inhibitor	Roche		II	$K_i = 0.1$ nM
Saxagliptin (BMS-477118)	Covalently bound product analogue	Bristol-Myers Squibb		III	$K_i = 0.45$ nM
PSN-9301	Reversible product analogue	Prosidion		II	Not available
NN-7201	Reversible nonpeptide heterocyclic inhibitor	NovoNordisk		-	Not available
Ddenagliptin (GSK-823093C)	-	GlaxoSmithKline		II	Not available
SYR-322	-	Takeda		III	Not available

Information mostly obtained from Pharmaprojects, V5.1 [201] and a recent review by McIntosh *et al.* [57].

-: Not known; DPP IV: Dipeptidyl peptidase IV; NDA: New drug application.

## 5. Drug development

The concept of DPP IV inhibitors was devised as a method of preventing DPP IV-mediated degradation of GLP-1 and GIP to extend their insulin-releasing activity. However, when GLP-1 and GIP were infused into rats at physiological concentrations, > 50% was truncated within 2 min [13]. Crucially, these truncated metabolites appeared to be absent after infusions into DPP IV-deficient animals [13]. Therefore, it was concluded that DPP IV was the primary inactivating enzyme of GIP and GLP-1 *in vivo*, and DPP IV inhibition was proposed as a potential strategy to prevent physiological inactivation of these incretins. Similarly, mice and rats deficient in DPP IV activity have an increased proportion of intact GLP-1 and GIP compared with truncated forms [52,54]. Studies in isolated perfused porcine ileum revealed that ~ 50% of released GLP-1 was rapidly truncated and that the application of a DPP IV inhibitor substantially increased intact levels of GLP-1 possibly to > 80% [55].

A wide range of DPP IV inhibitors have been developed and the structures and characteristics of several of these have recently been reviewed [56-59]. Table 3 lists various DPP IV inhibitors in development and their specificity ( $K_i$ ) or inhibitory coefficient ( $IC_{50}$ ). Many DPP IV inhibitors are classed as either reversible product analogues, covalently bound product analogues or reversible nonpeptide heterocyclic inhibitors (see Table 3). Novartis and Merck have tablet

formulations of DPP IV inhibitors in advanced Phase III clinical trials.

## 6. Current research goals

The current research goals in this field are:

- to review the specificity, selectivity, safety and efficacy of DPP IV inhibitors now in development
- to ascertain whether DPP IV inhibitors derive their anti-diabetic effect via mechanisms other than prevention of incretin hormone degradation
- to further investigate the effects of DPP IV inhibition on other systems and side effects resulting from cleavage of alternate substrates
- to ascertain whether a DPP IV inhibition strategy could be enhanced by the use of other inhibitors such as neutral endopeptidase 24.11 (NEP-24.11) inhibitors
- to evaluate effects of DPP IV inhibition on other systems, and potential resulting side effects

## 7. Scientific rationale

### 7.1 DPP IV in the pathophysiology of obesity and diabetes

There have been significant recent advances in our knowledge of the involvement of DPP IV in obesity and diabetes. Of

particular importance has been the application of gene 'knockout' to generate mice lacking DPP IV activity [51]. These mice are viable, healthy and their plasma possesses some residual cleavage of the substrate Gly-Pro-pNA. However, no significant N-terminal degradation of the incretin hormone GLP-1 was observed [51]. DPP IV-deficient mice have enhanced glucose tolerance and increased levels of plasma insulin, and it has been suggested that this results from higher levels of active GLP-1, and perhaps also active GIP. On a high-fat diet, mice lacking DPP IV gained less weight due to decreased food intake and increased energy expenditure [52]. DPP IV-deficient mice have improved insulin sensitivity and appear to resist hepatic lipid accumulation when fed a high-fat diet [52]. Findings in these DPP IV-knockout mice are in broad agreement with those of a rat strain harbouring mutant DPP IV, and strengthen the rationale for use of specific DPP IV inhibitors in Type 2 diabetes [53,54]. However, this evidence must be balanced against reports that receptor knockout mice *GIPR*<sup>-/-</sup> and *GLP-1R*<sup>-/-</sup> resist weight gain and show improved insulin sensitivity, thus indicating that loss of incretin action can have some similar long-term effects to loss of DPP IV activity [60]. The extent to which DPP IV inhibition could affect feeding behaviour and metabolite control through changes in the concentrations of other peptides (e.g., NPY, PYY or growth hormone-releasing hormone) remains to be established.

## 7.2 The effects of DPP IV inhibitors in animal models of diabetes

Several DPP IV inhibitors have been examined in diabetic rodents revealing glucose-lowering effects. In Zucker fatty rats, P32/98 (isoleucine thiazolidide) substantially decreased circulating DPP IV activity and improved glucose tolerance [59]. In addition, in Zucker rats, the inhibitor NVP-DPP728 amplified the early phase insulin response and restored glucose excursions to normal [61]. The effects of P32/98 and NVP-DPP728 contributed to increased levels of active GLP-1(7-36)amide. When the DPP IV inhibitor valine-pyrrolidide was coadministered with intravenous GIP the insulinotropic effect of GIP was improved, glucose clearance was enhanced and glucose excursions were reduced [62]. Another study examined the effects of vildagliptin (LAF-237) on plasma DPP IV activity, intact GLP-1, glucose and insulin after an oral glucose load over a 21-day period in insulin-resistant Zucker fatty rats [63]. Vildagliptin augmented glucose-stimulated circulating levels of intact, biologically active GLP-1 and exerted dose-dependent effects on DPP IV, glucose tolerance and  $\beta$ -cell function [63]. Vildagliptin had no effect on body weight in these studies.

Long-term, twice-daily P32/98 administration of Zucker rats for 3 months decreased body weight gain, but did not alter food intake [64]. Chronic P32/98 treatment improved glucose tolerance and increased insulin levels, but no significant differences in  $\beta$ -cell area or islet morphology were detected following the 12-week treatment period [64]. Strangely, fasting

DPP IV activity progressively increased over the treatment period and it was suggested that this might be a compensatory response to chronic DPP IV inhibition [64]. In streptozotocin-induced diabetic rats, long-term treatment with P32/98 led to increased weight gain, nutrient intake and insulin secretion and markedly improved glucose tolerance [65]. Furthermore, immunohistochemical studies suggested that P32/98 treatment enhanced islet neogenesis,  $\beta$ -cell survival and insulin biosynthesis, tentatively attributed to increased intact GLP-1 and GIP [65]. Mice fed on a high-fat diet, receiving NVP-DPP728 for 8 weeks, showed increased levels of intact GLP-1, improved glucose tolerance and increased glucose-stimulated insulin secretion, but there was reduced islet hyperplasia although expression of the glucose transporter, GLUT-2, was enhanced [66].

It appears that DPP IV inhibition as a strategy for improving glycaemic status is more effective in mild and moderate hyperglycaemic Type 2 diabetes than in severe diabetes. An investigation into the effects of DPP IV inhibition in the early and late stages of diabetes in *db/db* mice found that valine-pyrrolidide improved glucose tolerance at 6 weeks of age, but did not at 23 weeks of age [67]. These data suggest that DPP IV inhibitors may be useful as an early intervention strategy to address impaired glucose tolerance and the early stages of Type 2 diabetes [67].

Recent evidence suggests that DPP IV inhibition prevents degradation of the intestinotropic hormone, GLP-2, leading to enhanced small bowel weight [68]. Because GLP-2 is considered a potential treatment for colitis and short bowel syndrome, it has been suggested that DPP IV inhibitors could assist in the treatment of these gastrointestinal diseases [69,70].

## 8. Competitive environment

### 8.1 DPP IV inhibitors in Type 2 diabetes

So far, few clinical studies with DPP IV inhibitors in Type 2 diabetic patients have been reported (Table 4). A preliminary account of acute administration of P32/98 (60 mg) in non-diabetic and Type 2 diabetic subjects receiving other anti-diabetic therapies noted improved oral glucose tolerance associated with increased concentrations of intact GLP-1 [71]. A study giving NVP-DPP728 (100 mg t.i.d. and 150 mg b.i.d.) for 12 weeks to diet-treated Type 2 diabetic patients reduced fasting (by 1 mmol/l) and postprandial (by 1.2 mmol/l) blood glucose. Insulin concentrations were marginally reduced, possibly reflecting the reduced glycaemia, and body weight was unchanged [72]. NVP-DPP728 was a short-acting inhibitor, and has been discontinued and superseded by the longer-acting vildagliptin.

Administration of vildagliptin (100 mg/day) for 4 weeks to diet-treated Type 2 diabetic patients reduced fasting and postprandial plasma glucose (by 0.7 and 1.4 mmol/l, respectively) without a measurable change of insulin. Active GLP-1 (GLP-1[7-36]amide) concentrations were approximately doubled (postprandial concentrations rose to ~ 8 pmol/l

Table 4. DPP IV inhibitors studied in Type 2 diabetic patients.

First author	Year published	Agent tested	Other antidiabetic treatment	Duration (weeks)	n	Study design	Effect on glycaemic control
Ahren [72]	2002	NVP-DPP728 (100 mg t.i.d., 150 mg b.i.d.)	Diet only	4	93	RDBPC	↓ FBG 1 mmol/l ↓ PBG 1.2 mmol/l
Ahren [73]	2004	Vildagliptin 100 mg/day	Diet only	4	37	RDBPC	↓ FPG 0.7 mmol/l ↓ PPG 1.4 mmol/l
Ahren [74,75]	2004 2005	Vildagliptin 50 mg/day	Metformin	52	107	RDBPC*	↓ FPG 1.1 mmol/l ↓ PPG 2.4 mmol/l ↓ HbA1c 1.1%
Ristic [77]	2005	Vildagliptin 25 – 100 mg/day	Diet only	12	279	PC	↓ FPG 0.54 mmol/l† ↓ PPG 0.89 mmol/l† ↓ HbA1c 0.53%‡
Mari [79]	2005	Vildagliptin 200 mg/day	Diet only	4	20	RDBPC	↓ FPG 1.2 mmol/l
Dejagers [76]	2006 Abstract	Vildagliptin 100 mg/day	Diet only	52	526	RDBC	↓ HbA1c 1.0%
Ascher [83]	2006 Abstract	Sitagliptin 100 mg/day	Diet only	24	741	RDBPC	↓ HbA1c 0.79%
Karasik [84]	2006 Abstract	Sitagliptin 100 mg/day	Metformin	24	701	RDBPC	↓ HbA1c 0.65%
Herman [81]	2005 Abstract	Sitagliptin 25 – 100 mg/day	Diet only	12	552	RDBPC	↓ FPG 1.7 mg/ml (0.95 mmol/l)† ↓ HbA1c 0.7%‡
Brazg [82]	2005 Abstract	Sitagliptin 100 mg/day	Metformin	4	28	RDBPC	↓ FPG 2.0 mg/ml (1.1 mmol/l)

\*For first 12 weeks; †For the 100 mg/day dose; ‡Decrease.  
b.i.d.: Twice daily; FBG: Fasting blood glucose; FPG: Fasting plasma glucose; PBG: Postprandial blood glucose; PC: Placebo controlled; PPG: Postprandial plasma glucose; RDBC: Randomised double-blind comparator; RDBPC: Randomised double-blind placebo controlled; t.i.d.: Three times daily.

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before and 16 pmol/l after treatment), whereas glucagon concentrations were reduced [73]. A 52-week trial in which vildagliptin (50 mg/day) was added to metformin therapy in Type 2 patients noted a sustained decrease in HbA1c by 1.1% compared with continuation of metformin alone [74]. The plasma insulin response to a standard meal was slightly higher in those receiving the vildagliptin together with metformin, but there were no significant changes in body weight, fasting triglycerides and low- or high-density lipoprotein cholesterol. Using data from this study it was estimated that addition of vildagliptin to metformin therapy improved  $\beta$ -cell function and increased insulin sensitivity [75]. A preliminary (abstract) account of a 52-week comparator study between vildagliptin 100 mg/day and metformin 2000 mg/day in diet-treated Type 2 patients has suggested a slightly greater reduction in HbA1c with metformin (-1.4%) than vildagliptin (-1.0%) [76].

A dose-response study in diet-treated Type 2 diabetic patients receiving vildagliptin 50 and 100 mg/day for 12 weeks showed reductions in HbA1c (0.43 and 0.4%, respectively), but a lower dose (25 mg/day) did not exert a significant effect [77]. Postprandial insulin concentrations were significantly increased with the 100 mg/day dose of vildagliptin, and this dose improved  $\beta$ -cell function (but not insulin sensitivity), as indicated by homeostasis model assessment. During an intravenous glucose tolerance test, vildagliptin (100 mg/day for 12 weeks) increased the acute insulin response by more than threefold in a small group of Type 2 patients [78]. Insulin concentrations measured in a 4-week study of Type 2 diabetic patients receiving vildagliptin 200 mg/day were applied to a different mathematical model from which it was also concluded that  $\beta$ -cell function was improved [79]. In this study, vildagliptin more than doubled the concentrations of intact GLP-1 and GIP, and glucagon concentrations were halved.

Clinical studies in nondiabetic subjects have been reported in preliminary (abstract) form for sitagliptin (MK-0431), showing tolerability and general pharmacokinetic and pharmacodynamic properties [64]. In a 12-week trial in diet-treated Type 2 patients, citagliptin 25 – 100 mg/day improved glycaemic control in a dose-related manner (decrease HbA1c by 0.7% with 100 mg/day dose), with no change in body weight [81]. A 4-week study in which sitagliptin was added to metformin-treated Type 2 diabetic patients also noted an improvement in glycaemic control without a change in body weight [82]. Preliminary data (abstracts) from Phase III trials indicate that citagliptin (100 mg/day for 24 weeks) reduced HbA1c by 0.79% in diet-treated Type 2 diabetes patients [83], and by 0.65% when added to metformin [84].

General features emerging from clinical studies with DPP IV inhibitors in Type 2 diabetes indicate a modest, but sustained, reduction in hyperglycaemia without weight gain. Insulin concentrations may be unchanged or marginally increased, but irrefutable evidence of improved  $\beta$ -cell function is still required and there are insufficient data to determine whether DPP IV inhibitors can independently

improve insulin sensitivity. DPP IV inhibitors have generally increased the amount of active GLP-1 and reduced circulating glucagon levels. Encouragingly, there has been no serious hypoglycaemia in any of the reported trials. The ability of therapeutic doses of DPP IV inhibitors to produce almost complete inhibition of DPP IV activity for at least 12 h has been confirmed in nondiabetic and Type 2 diabetic subjects [73,80]. No serious or consistent adverse events have been associated with DPP IV inhibition so far.

Recent evidence has emerged to suggest that GLP-1 could exert vascular effects that provide some protection against ischaemic/reperfusion injury to the myocardium and improve ventricular recovery and function in heart failure [85,86]. Although it is premature to extrapolate these effects to DPP IV inhibitors, this is an interesting new area.

### 8.2 DPP IV inhibitors in Type 1 diabetes

Incretin hormone secretion is normal in Type 1 diabetes [87], and GLP-1 administration can improve glycaemic control in Type 1 diabetes [88]. This has prompted questions concerning possible benefits of DPP IV inhibitors together with insulin therapy in Type 1 diabetes. In the absence of islet  $\beta$ -cells the effects of GLP-1 in Type 1 diabetes are likely to reflect mainly decreased glucagon and decreased gastric emptying, and possibly satiety. Indeed, glucagon suppression has been demonstrated during use of DPP IV inhibitors, which could provide a mechanism of therapeutic value in Type 1 diabetes. It is unknown whether the potential for GLP-1 to increase  $\beta$ -cell neogenesis and division, and to reduce  $\beta$ -cell apoptosis in diabetic rodents could benefit human Type 1 diabetes, especially while the autoimmune destructive process remains highly active. Possible effects of DPP IV inhibitors to reduce T-cell proliferation and chemotaxis have been mooted as a possible means to counter the autoimmune disease. However, evidence for such an approach is very preliminary, and nonspecific interference with the immune system must carry important safety caveats.

### 8.3 Effects of antidiabetic drugs in relation to DPP IV activity

Metformin, a widely used antidiabetic biguanide drug, has been reported to reduce DPP IV activity in Type 2 diabetic patients and diabetic animal models [89-91], although this is not a universal finding [92,93]. A study employing 20 obese nondiabetic human males found that metformin treatment for 14 days increased active levels of GLP-1 [89]. In addition, the degradation of GLP-1(7-36)amide to GLP-1(9-36)amide by pooled human plasma was inhibited by metformin, thus offering an explanation for the higher levels of active GLP-1 [89]. Furthermore, metformin therapy inhibited DPP IV activity in Type 2 diabetes [91]. Pioglitazone (a thiazolidinedione), which has antidiabetic effects mediated through PPAR $\gamma$ , has been reported to lower serum DPP IV activity [90]. Little information is available at present regarding possible effects of other commonly used antidiabetic drugs on DPP IV activity.

## 9. Potential development issues

### 9.1 The selectivity of DPP IV inhibitors

Identification of several new members of the DPP IV family has reopened the issues of selectivity of current DPP IV inhibitor compounds. Inhibitors previously thought to be specific for DPP IV could in fact be inhibitors of other members of the DPP IV enzyme family. Thus, a re-evaluation of the selectivity of DPP IV inhibitors is in progress. Val-boro-Pro is one example of a DPP IV inhibitor that is not sufficiently selective for DPP IV and may also inhibit FAP [69]. Val-boro-Pro also appears to inhibit DPP 8, 9 and II [70].

The selectivity of several DPP IV inhibitor compounds for DPP IV, FAP, DPP 8, DPP 9 and DPP II enzymes has recently been tested and reviewed [70]. Identification of a DPP IV-, DPP 8/9- and DPP II-selective compound allowed an evaluation of potential toxicity and tolerability of each type of inhibition. In rats, the DPP 8/9 inhibitor produced alopecia, thrombocytopenia, reticulocytopenia, multiorgan histopathological changes, enlarged spleen and mortality. The same inhibitor produced gastrointestinal toxicity in dogs. The DPP II-selective inhibitor produced reticulocytopenia in rats. Conversely, the DPP IV-selective inhibitor appeared to cause no toxicity [70]. However, 4 weeks of treatment with DPP IV inhibitor, p32/98, produced evidence of adverse effects on the lungs, thrombocytopenia, ataxia, seizures, convulsions, tremor and diarrhoea [70]. Off-target inhibition was cited as a possible reason for these toxicities. DPP 8/9-selective inhibition attenuated T-cell activation suggesting involvement of these enzymes in the immune system. Current information suggests that DPP IV-selective compounds, particularly those with no inhibition of DPP 8/9, are more likely to be safer, better tolerated and more efficacious.

### 9.2 Potential cautions of DPP IV inhibition

As noted earlier, although there is only limited information concerning exposure of human subjects to DPP IV inhibitors, no serious safety issues have emerged. However, there is a theoretical potential to interfere with the actions of many regulatory peptides and immune processes (Table 2). DPP IV-knockout and -deficient rodents have not shown adverse events [53], although this does not preclude actions of the DPP IV inhibitors that are independent of DPP IV inhibition.

Applying conventional risk-benefit assessment criteria to existing published clinical data for DPP IV inhibitors may be premature, but it is noteworthy that although improvements in HbA1c are modest, they have been sustained without contentious effects on standard blood biochemistry or electrocardiogram results, and discontinuation rates have been low.

## 10. Expert opinion and conclusion

DPP IV inhibitors are a potential new oral drug class for the treatment of Type 2 diabetes. They appear to act at least in

part by increasing the physiological incretin effect, although other effects may also be involved. Although there are several DPP IV inhibitors that demonstrate appropriate therapeutic potential, the identification of new members of the DPP IV enzyme family has raised new issues over their selectivity and, therefore, their risk-benefit ratio. Nonselective DPP IV inhibition appears to increase the risk of adverse events. There are unanswered questions concerning the potential effects of DPP IV inhibitors on the immune system and more data are needed to assess the involvement of other incretin-degrading enzymes, particularly NEP-24.11. The potential to combine the therapeutic effects of DPP IV and NEP-24.11 inhibitors awaits further evaluation.

Although DPP IV is an important inactivator of the incretin hormones *in vivo*, few investigations have fully examined the action of other enzymes that metabolise these peptides. NEP-24.11 (EC 3.4.24.11), otherwise known as neprilysin, is a widespread membrane-bound zinc metallopeptidase with a broad substrate specificity. Multiple degradation fragments have been noted following the incubation of GLP-1 with purified human neutral endopeptidase (NEP-24.11) [94] and with RINm5F plasma membranes [95] containing NEP-24.11 activity. GIP is also degraded by NEP-24.11 [95]. NEP-24.11 is found in high concentrations in the kidney, where it is involved in the renal clearance of peptide hormones. Although DPP IV inhibition improves the insulinotropic and anti-hyperglycaemic activity of GLP-1, these effects can be further enhanced by concomitant NEP-24.11 inhibition [96]. NEP-24.11 accounted for up to 50% of the degradation of GLP-1. Because the mechanism of action of DPP IV inhibitors is based on their ability to preserve active levels of endogenous GLP-1 and GIP, it remains to be seen whether this strategy alone will be sufficient for potent and sustained glucose-lowering efficacy, or whether an additional inhibitor of NEP-24.11, such as a candoxatril-related agent, will provide added efficacy. Further investigation of NEP-24.11 degradation of incretin hormones is needed.

### 10.1 DPP IV inhibition versus incretin mimetics

Peptide incretin analogues and mimetics that display resistance to DPP IV degradation offer a protracted duration of action. Incretin mimetics behave as the incretins do, with defined biological actions and defined target tissues, as indicated by expression of the GIP and GLP-1 receptors. This is in contrast to DPP IV inhibitors, which have a broad substrate specificity, and thus DPP IV inhibitors will likely have multiple effects on multiple systems. Compared with DPP IV inhibitors, the incretin hormones appear to lower plasma glucose via a number of mechanisms beyond increased glucose-dependent insulin secretion and reduced glucagon levels. For example, the incretin hormones may improve glucose uptake and use, while suppressing gastric emptying, and increasing feelings of satiety [1].

The first of the incretin hormone mimetics, exenatide, was launched in the US in June 2005, and other GLP-1-like

compounds are expected [19]. Although incretin mimetics appear to have had a 'head start', several DPP IV inhibitor compounds are now in Phase III clinical trials. Assuming successful approval of DPP IV inhibitor drugs, important questions will concern which class of compounds, DPP IV inhibitors or incretin mimetics, are more efficacious, convenient, tolerable, safe and suitable for which patient groups. An advantage offered by DPP IV inhibitors is oral administration, compared with subcutaneous injection of incretin mimetics. Although the incretins are usually rapidly filtered by the kidney, strategies to circumvent renal filtration are now being incorporated into the design of incretin hormone analogues. This could substantially extend their duration of action and reduce administration frequency. Although there is some evidence that DPP IV inhibitors cause histological changes to islets [65], this is now inconclusive [64]. In addition, whether DPP IV inhibitors have antidiabetic effects that are independent of incretin activity or indeed independent of DPP IV inhibition remains unclear [75,97,98]. A recent report suggests that GLP-1(9-36)amide (obtained through DPP IV cleavage of GLP-1) lowers postprandial glycaemia independently of effects on insulin secretion, glucagon secretion or the rate of gastric emptying [50], whereas another study suggests that GLP-1 metabolite formation by DPP IV does not affect glycaemic control in Type 2 diabetes [14]. Whether, GLP-1 and GIP analogues or DPP IV inhibitors exert proliferative and protective effects on the pancreatic  $\beta$ -cell, improving cell mass and insulin biosynthesis during treatment in human subjects is not established (Table 1) [1].

In view of the many biological peptides that are susceptible to DPP IV (Table 2), and the importance of these peptides in a wide range of metabolic, vascular, neural, immunological and other physiological control processes, the question of possible adverse effects remains a more prominent issue for DPP IV inhibition than administration of incretin mimetics. However, it is noteworthy that DPP IV elimination in animal

models and use of specific DPP IV inhibitors in clinical trials so far has not exposed any obvious serious detrimental effects. The prospect of preserving  $\beta$ -cell function and mass with either an incretin mimetic or a DPP IV inhibitor favours use as part of an early intervention strategy before the  $\beta$ -cell population is inordinately damaged and depleted. Whether this is better achieved by short-acting reversible specific DPP IV inhibition or more protracted specific DPP IV inhibition remains to be established. Potential theoretical advantages of reducing unwanted side effects by short-acting inhibitors 'that lose activity overnight when the patient is not feeding' have yet to be demonstrated in a clinical setting.

It remains a possibility that incretin hormones could be given in combination with inhibitors of DPP IV (and NEP-24.11). However, a difference between administration of a GLP-1 analogue and a DPP IV inhibitor is that the concentration of the analogue will be much greater than the endogenous incretin. While this accentuates a wide range of physiological GLP-1-mediated effects, it also introduces side effects such as nausea. DPP IV inhibition will sustain levels of the active endogenous incretins for longer, but may not raise levels greatly above the physiological maximum. Indeed, production of persistently increased active incretin levels by DPP IV inhibition might generate a feedback loop to reduce endogenous incretin production [99] or create resistance to the incretins [100]. Because endogenous active GLP-1 levels are generally lower in Type 2 diabetes than in nondiabetic individuals [48], it may be questioned whether the increased active incretin levels achievable by DPP IV inhibition would on their own be sufficient to produce adequate therapeutic efficacy. Nevertheless, as noted in clinical trials so far, DPP IV inhibitors have sustained substantial improvements in glycaemic control in Type 2 diabetes with apparent tolerability and few adverse effects. Whether these agents are exerting their antihyperglycaemic effects through mechanisms additional to prolonged endogenous incretin hormone activity is, therefore, a possibility deserving attention.

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## Website

201. <http://www.pjbpubs.com/pharmaprojects/index.htm>

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