Monthly Focus: Endocrine & Metabolic

# Inhibitors of dipeptidyl peptidase IV: a novel approach for the prevention and treatment of Type 2 diabetes?

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Inhibitors of the enzyme dipeptidyl peptidase IV (DPP IV) are of increasing interest to both diabetologists and the pharmaceutical industry alike, as they may become established as the next member of the oral antidiabetic class of therapeutic agents, designed to lower blood glucose and, possibly, prevent the progressive impairment of glucose metabolism in patients with impaired glucose tolerance and Type 2 diabetes. DPP IV has become a focus of attention for drug design, as it has a pivotal role in the rapid degradation of at least two of the hormones released during food ingestion, a property that has warranted the design of inhibitor-based drugs. At the molecular level, DPP IV cleaves two amino acids from the N-terminus of the intact, biologically active forms of both so-called incretin hormones, glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide (formerly known as gastric inhibitory polypeptide), resulting in truncated metabolites, which are largely inactive. Inhibition of the enzyme, therefore, is thought to increase levels of the active forms of both incretin hormones, culminating in an increase in insulin release after a meal, in a fully glucose-dependant manner. DPP IV inhibitors combine several features of interest to the drug design process. They can be readily optimised for their target and be designed as low molecular weight, orally active entities compatible with once-daily administration.

**Keywords:** dipeptidyl peptidase, enteroinsular axis, enzyme inhibitor, glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, incretin, oral antidiabetic agent

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## 1. Introduction

Type 2 diabetes is a component of a disease cluster collectively known as the metabolic syndrome, comprising of a variety of disorders including glucose intolerance/insulin resistance, arterial hypertension, dyslipidaemia and obesity. It has emerged as one of the world's major debilitating diseases. A clear requirement for new and more effective drugs for the prevention and treatment of Type 2 diabetes is demonstrated by a combination of the following points:

- the well-reported escalation in the numbers of people suffering from this disease cluster
- an overtly unsatisfactory, and still surprisingly small, family of currently available treatments, the outcome of which leaves many patients with a reduced quality of life because of associated complications including cardiovascular problems, retinopathy, nephropathy and neuropathy
- a lack of available drugs for the prevention of the condition.

Expert Opinion

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Almost all of today's drug therapies for Type 2 diabetes have been a result of the serendipitous discovery of the antidiabetic activity of compounds whose precise mechanisms of action were obscure at the time of discovery and, in some cases, remain unresolved. This means that many of the currently available drugs have some clinically relevant side effects, which may have been avoided had rational drug design been possible. This picture may well now be set to change due to the emergence of a new treatment modality that has resulted from the rational design of a drug class based on the precise knowledge of the salient molecular target. It is not often that clinical proof of concept (CPOC) of an entirely new oral treatment modality emerges for a major debilitating disease. The inhibitors of dipeptidyl peptidase IV (DPP IV) for the treatment of Type 2 diabetes do, however, represent such a concept.

## 2. Dipeptidyl peptidase IV inhibitors as new oral antidiabetic drugs

Following promising results in preclinical studies, mainly in rodents, Ahrén and colleagues [1,2] reported CPOC for the use of selective and specific inhibitors of DPP IV to treat Type 2 diabetes. This enzyme is responsible for the rapid degradation of the body's so-called incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are integral components in the physiological control of insulin release and, therefore, in the regulation of blood glucose. Consequently, the inhibition of this enzyme will prevent the degradation of these important incretin hormones, leading to enhancement of their physiological effects.

### 2.1 Background

In the late 1980s, the newly discovered gastrointestinal hormone GLP-1 [3,4] was found to possess potent insulin-releasing abilities [4-7], spurring interest in using this peptide therapeutically to treat diabetic hyperglycaemia. In addition to its insulinotropic action, GLP-1 possesses a spectrum of activities (β-cell tropic/anti-apoptotic, glucagonostatic, appetite suppressing, gastric emptying rate reducing [8]), which makes it an apparently ideal antidiabetic agent. In the early 1990s, the first reports of its effects in patients with Type 2 diabetes appeared [9,10]. Remarkably, continuous infusion of GLP-1 normalised glucose levels (both fasting and postprandial) in these individuals [11-13], even in those with poorly controlled diabetes long after sulfonylurea secondary failure [14]. Single subcutaneous injections were, however, less effective and glucose concentrations were not normalised [15,16], whereas the effects of continuous subcutaneous infusion for 6 weeks in diabetic patients has subsequently been reported with very promising results [17]. Furthermore, buccal administration of the peptide showed it to be efficient in healthy subjects and in patients with Type 2 diabetes [18,19]. The surprising and unexpected ineffectiveness of a single administration of GLP-1 turned out to be due to a rapid inactivation of the peptide *in vivo*. An earlier published meeting abstract had indicated that GLP-1 could be N-terminally degraded by plasma *in vitro* [20], and subsequently, DPP IV was shown to be capable of mediating such cleavage *in vitro* [21]. Later studies suggested that DPP IV was likely to play a major role in regulating the metabolic fate of GLP-1 *in vivo* [22-24], and it was also reported that DPP IV mediates the inactivation of GIP [24,25]. These observations were confirmed and extended to include the endogenous peptide in studies in which the enzyme activity was selectively deleted [25-28].

The principle of harnessing the endogenous incretin hormones to treat Type 2 diabetes by using DPP IV inhibitors was first described in 1995 [23]. The rationale is that by inhibiting DPP IV activity, levels of the intact, biologically active forms of both hormones will be increased into the range shown to be therapeutically useful [29]. The beauty of this approach is that the body's own normal homeostatic mechanisms are enhanced. Thus, the inhibitor can be given and will result in increased levels of incretin hormones and, therefore, insulin will be released only when the body needs it (i.e., mainly in relation to food intake because the incretin hormones are released in response to the presence of nutrients in the small intestine) [3]. Moreover, because the insulin-releasing effects of the incretins are glucose-dependent, insulin secretion is only enhanced when blood glucose levels rise [30], and the risk of hypoglycaemia is minimal. The concept is also supported by results from animals with a genetic deletion of DPP IV (the CD26 knockout mouse [27]) or with a mutant, catalytically inactive DPP IV molecule (the Fischer rat [31]), which have increased active GLP-1 and probably GIP levels, and improved glucose tolerance.

### 2.2 Dipeptidyl peptidase IV inhibitors as antihyperglycaemic agents

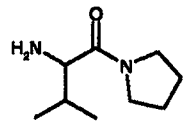
A number of acute studies in animal models have exemplified the beneficial effects of DPP IV inhibitors on glucose intolerance [32-34] and the results of chronic treatment have recently appeared [35-39]. These studies confirm that the effects of DPP IV inhibitors mainly reflect the known pharmacology of GLP-1. Although a reduction in food intake and body weight was seen in response to these drugs in two rat studies [35,36] it would appear that most early reports are suggestive of little or no effect on body weight, which is in contrast to GLP-1. However, even body weight neutrality, if proven clinically, would distinguish DPP IV inhibitors from the currently available therapies, which increase body weight and probably exacerbate the vicious cycle of events comprising Type 2 diabetes. Encouragingly, the first preliminary communication of a 1-year clinical trial with the inhibitor LAF-237 given in combination with metformin reported no weight gain over the study period [40]. The preclinical studies also suggest that insulin sensitivity may be improved by chronic DPP IV inhibition, possibly as a result of chronic lowering of blood glucose and a reduction of a phenomenon called glucose toxicity rather than as a direct effect of the drug itself, reflecting one of

the observations noted in the 6-week study of GLP-1 infusion [17]. Intriguingly, CD26 knockout mice and DPP IV deficient Fischer rats are protected against diet-induced obesity and insulin resistance [41,42], suggesting that in the longer term, DPP IV inhibition may affect body-weight control and energy homeostasis. DPP IV inhibitor-mediated preservation of the body's endogenous GLP-1 may also enhance the long-known effects of GLP-1 in terms of β-cell rescue/prevention of apoptosis, indicating a use for this drug class in the prevention of Type 2 diabetes [29] and, as a related property, in the prevention of the worsening of the disease. Indeed, chronic treatment with a DPP IV inhibitor preserved islet function in diabetic mice [39] and improved β-cell survival and islet cell neogenesis in streptozotocin diabetic rats [38].

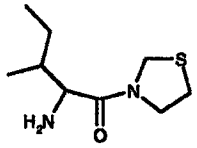
The promise held by DPP IV inhibitors appears to be seen in the first trials in patients with Type 2 diabetes [1,2]. It is particularly noteworthy that these studies show that, with a DPP IV inhibitor, lowering of both fasting and postprandial blood sugar is possible. This is important because increased fasting and postprandial blood sugar are both thought to contribute to elevated glycosylated haemoglobin (HbA1c) levels, a key variable reflecting the average glycaemic levels over several months, known to be associated with the development of debilitating complications of Type 2 diabetes.

The publication of data from studies of ≥ 3 months in length, and from more substantially diabetic patients are awaited in order to judge both efficacy and incidence of side effects that may be expected from DPP IV inhibitors because the patients in both of Ahrén's studies were still in the early stage of the disease and the treatment was given for only 4 weeks. However, there is reason to be optimistic. Preclinical studies (e.g. [35]) in diabetic rodents show that the effects of DPP IV inhibitors on glucose tolerance become more marked as the dosing period continues, so that the efficacy after 4 weeks in the human studies reported thus far may well underestimate the eventual steady-state effect of DPP IV inhibition on fasting blood glucose in particular. Nevertheless, despite the relatively short treatment period studied, a significant effect on HbA1c was seen. The starting value of 7.4% for HbA1c, as was seen in the clinical studies of DPP IV inhibition [1,2], reflects the mild diabetes of this patient cohort. This, on the other hand, means that any fall in HbA1c would be small, in spite of significant improvements in glycaemic control, so it is all the more pleasing to see a significant reduction in HbA1c (of 0.5% to 6.9%) in these studies. The effect of DPP IV inhibition is expected to become less marked as blood glucose returns to normal (the so-called glucose dependency [30]), which is important in limiting one of the most serious of all side effects of some current therapies (i.e., hypoglycaemia). Recently reported preliminary findings from longer-term (12 weeks) monotherapy with LAF-237 [43] show sustained reductions in HbA1c (from a baseline value of 8 to reach 7.4% by the end of the study), suggesting that tachyphylaxis does not develop, with those patients with higher baseline (starting) HbA1c levels showing the greatest reduction; those with a starting level between 7 and 8% fell 0.7% compared with placebo treatment, while those between 8 and 9.5% declined 1.2% relative to placebo. Furthermore, early reports from 3- and 12-month combination therapy of LAF-237 with metformin also indicate that significant reductions in HbA1c levels are maintained [40,44]. HbA1c levels in those patients taking both LAF-237 and metformin were reduced from a starting value of 7.8 to 7.2% in the first 3 months [44], and this effect was sustained during an extension of the study (HbA1c at 7.3% after 1 year) [40]. In contrast, after an initial fall, HbA1c levels began to increase in the patients taking metformin alone, so that by the end of 3 months it was back to baseline (7.9%) [44], and continued to increase in the extension period to reach 8.4% by 1 year [40]. However, treatment of even impaired glucose tolerance is important for reducing the cardiovascular consequences of the metabolic syndrome [45,46].

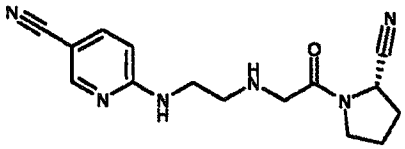
One issue that is often raised is the question of adverse side effects, arising either as a consequence of inhibiting the catalytic activity of a molecule that has other functions in addition to degrading regulatory peptides, or because of the effect on multiple substrates of DPP IV. Other functions of DPP IV [47] include a role in the immune system, where it has the capacity to serve as a costimulatory surface molecule influencing T-cell activity, although in this context it is uncertain whether the catalytic activity *per se* is required. Moreover, there is now known to be a family of closely related enzymes, which share DPP IV-like catalytic activity, including the recently identified DPP 8 [48] and DPP 9 [49], which may be responsible for some of the functions previously attributed to DPP IV itself. It is possible that some of the described DPP IV inhibitors (Figure 1 and Table 1) may not be completely selective for DPP IV, and may influence the activity of other enzymes, such as DPP 8 and DPP 9. In this context, early data suggest that DPP IV-selective inhibitors do not affect *in vitro* T-cell activation, while a DPP 8/9 inhibitor and a nonselective inhibitor do [50], suggesting that previously reported immunological effects of some DPP IV inhibitors could have been due to their effect on DPP 8 or DPP 9, rather than on DPP IV itself. Other preliminary *in vivo* studies, reporting that selective inhibition of DPP 8/9 is associated with profound toxicities (rodents) or adverse side effects (dogs) whereas selective inhibition of DPP IV is not [51], suggest that the issue of inhibitor selectivity must be addressed. The other question relates to whether DPP IV inhibition may be problematic in terms of adverse side effects, which theoretically may be expected from inhibition of an enzyme with apparently multiple substrates, via both the accumulation of adverse substrates and the inhibition of the formation of beneficial products (reviewed by Mentlein [52] and Lambeir *et al.* [47]). On one hand, inhibition of the multiple substrates of an enzyme may contribute to efficacy (e.g., inhibitors of angiotensin-converting enzyme [ACE] possess efficacy due to the modulation of at least two substrates, angiotensin I and bradykinin). On the other hand, it is possible that changes in the concentrations of



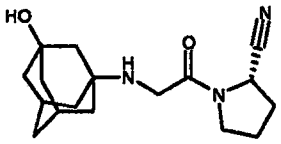
Valine-pyrrolidide



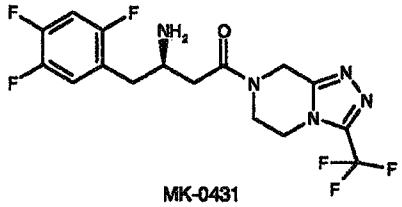
Isoleucine-thiazolidide



NVP-DPP-728



LAF-237



MK-0431

**Figure 1. Structures of dipeptidyl peptidase IV inhibitors.** Data are taken from [26] (valine-pyrrolidide), [32] (isoleucine-thiazolidide), [98] (NVP-DPP-728 and LAF-237) and [72] (MK-0431).

**Table 1. Selectivity data for dipeptidyl peptidase IV inhibitors.**

| | $IC_{50}$ / $K_i$ (nM) | | | | | |
|---|---|---|---|---|---|---|
| | DPP IV | QPP/DPP II | PEP | FAPα | DPP 8 | DPP 9 |
| Valine-pyrrolidide | 255* | 26,000* | > 10,000 | ? | ? | ? |
| Isoleucine-thiazolidide (P32/98) | 126* | 7,000* | ? | ? | ? | ? |
| NVP-DPP-728 | 22 | 110,000 | 190,000 | ? | ? | ? |
| LAF-237 | 3.5 | > 500,000 | 210,000 | ? | ? | ? |
| MK-0431 | 18 | > 100,000 | > 100,000 | > 100,000 | 48,000 | > 100,000 |

Data are taken from [97] (valine-pyrrolidide and isoleucine-thiazolidide), [98] (NVP-DPP-728 and LAF-237) and [72] (MK-0431). *These values are $K_i$.
DPP: Dipeptidyl peptidase; $IC_{50}$: Median inhibitory concentration; FAPα: Fibroblast activatoin protein-α (seprase); $K_i$: Inhibition constant; PEP: Prolylendopeptidase (prolyloligopeptidase/postproline cleaving enzyme); QPP: Quiescent cell proline dipeptidase.

multiple endogenous substrates may be a source of unwanted side effects. It is critically important that, when considering possible substrates for DPP IV, one differentiates between substrates that are known to be physiologically relevant (i.e., endogenous substrates) and those that have been identified in assays *in vitro* where often high concentrations of substrates are offered to the enzyme in possibly aphysiological conditions. Measurement of endogenous levels of substrates, *in vivo*, after DPP IV inhibition, provides the most relevant method for judging which substrates are relevant to the action of DPP IV inhibitors. Moreover, we should remember that even physiologically relevant substrates may also have multiple routes of degradation, so that the inhibition of DPP IV will not prevent the metabolism of these substrates via alternative routes. Ultimately, only careful assessment of efficacy versus side effects will determine whether enzyme inhibitors will become established therapy for any given indication. It is comforting to see that the incidence of pruritis, a possible consequence of substance P accumulation, was only transiently experienced, and then only in the study with the inhibitor NVP-DPP-728 [1] and not in those patients receiving LAF-237 [2], suggesting that some other DPP IV substrates may find alternative routes of degradation once DPP IV becomes inhibited for a longer period, or that this

was a compound-specific, rather than a class-specific, effect. So far, few other side effects have been reported after 4 weeks of administration of LAF-237 and NVP-728 to man [1,2], and this also seems to be the case regarding the preliminary reports on 12-week LAF-237 monotherapy [43], and 3-month and 1-year combination therapy of LAF-237 with metformin [40,44]. Currently available information suggests, therefore, that the drug class appears to be well-tolerated and those side effects reported have been minor, transient and compound, rather than class specific.

The fact that multiple substrates for DPP IV are thought to exist may also help explain the positive effects of these drugs on glycaemia. When we consider that islet function is regulated not only by substrates and incretin hormones but also by nerves [53], it can be seen how modulation of other neuropepetides may contribute to efficacy. Sympathetic, parasympathetic and sensory nerves are known to innervate the islets. These nerves not only harbour the classical neurotransmitters acetyl choline and noradrenaline but also several neuropeptides are localised to islet nerve terminals. These neuropeptides include pituitary adenylylate cyclase-activating polypeptide (PACAP), gastrin-releasing peptide (GRP) and vasoactive intestinal polypeptide (VIP) in parasympathetic nerve terminals, galanin and neuropeptide Y (NPY) in sympathetic nerve terminals and calcitonin gene-related polypeptide (CGRP) in sensory nerve terminals, all of which have been shown to affect islet function [53]. Biochemical studies have shown that several of these islet neuropeptides are substrates for DPP IV and, therefore, DPP IV inhibition may be expected to prolong their half-lifes and consequently their action. The neuropeptides shown to be substrates for DPP IV include PACAP, VIP and GRP [54,55], and for some of them at least, DPP IV seems to be relevant physiologically (e.g., PACAP [55]). It is, therefore, of relevance that a main function of these neuropeptides is to stimulate insulin secretion in a glucose-dependent manner [53,56]. Furthermore, both PACAP and GRP stimulate cellular proliferation [57-59] and inhibit apoptosis [60], whereas PACAP has also been shown to prevent the development of streptozotocin-induced diabetes in rats [61] and to reduce the hyperglycaemia in models of impaired glucose tolerance and Type 2 diabetes in rodents [62]. Therefore, an additional advantage of DPP IV inhibition could be that the islet effects of these neuropeptides are augmented, which may contribute to the beneficial effect. In fact, their antidiabetic action may explain the experience of the clinical studies undertaken so far, in that DPP IV inhibition [1,2,40,43,44] seems as efficient as GLP-1 analogues [63-66], at the doses reported, in spite of GLP-1 concentrations being increased to a lower degree than possible after analogue administration. However, it must be noted that no direct head-to-head comparisons of the maximal antihyperglycaemic effects (i.e., efficacy) have yet been reported. Furthermore, the contribution of these neuropeptides to the beneficial effect of DPP IV inhibition in relation to the contribution of GLP-1 remains to be established. After acute DPP IV inhibition, at least, all of the beneficial effects on glucose tolerance appear to be mediated via GLP-1 and GIP receptor signalling, as the glucose-lowering actions of DPP IV inhibitors were eliminated in the double incretin receptor knockout mouse, in contrast to both the single incretin receptor knockout mice, in which DPP IV inhibition does lower glucose and increase plasma insulin levels [67]. It remains to be seen whether after longer-term DPP IV inhibition, the potential neuropeptide substrates of DPP IV may contribute. Furthermore, it will only be possible to judge their contribution when analytical techniques are developed, which allow the measurement of endogenous levels of the intact and DPP IV-truncated derivatives of these neuropeptides.

## 3. What may we regard as the key properties of dipeptidyl peptidase IV inhibitors?

### 3.1 Efficacy as monotherapy: glycosylated haemoglobin lowering

It is obvious that any newly emerging oral antidiabetic (OAD) should prove effective versus placebo as monotherapy. This is difficult to judge from the published 4-week trials that are available because steady-state reductions of HbA1c are only likely to be measurable after ≥ 3 months. It should be taken into account that the reduction of HbA1c seen after 4 weeks of treatment with the inhibitors NVP-DPP-728 and LAF-237, of 0.5% [1,2], will probably end up being a significant underestimate of efficacy on a long-term basis. When judging efficiency it is important to emphasise that end-point HbA1c values correlate to the starting value and that placebo comparisons should always be made. It should also be emphasised that any reduction in HbA1c is limited when the starting value is itself low. It is, therefore, encouraging that the preliminary results after 12-week monotherapy with LAF-237 seem to suggest that, while all patients show improvements in HbA1c levels, greater reductions are achievable by those with higher starting values [43].

At present, based on the available information, it is likely that DPP IV inhibitors will have equal efficacy as monotherapy as existing OADs, and may be competitive versus the thiazolidinedione (TZD) insulin sensitisers.

### 3.2 Efficacy in more advanced forms of Type 2 diabetes

A long-term effect in advanced Type 2 diabetes, when insulin secretion is severely impaired, would rely on a significant effect of DPP IV inhibitors in reducing glucose excursions to a mixed meal, presumably as a result of GLP-1-induced lowering of glucagon secretion, and possibly a delay in gastric emptying. To date, there have been no clinical reports of DPP IV inhibitors affecting gastric emptying but reduced glucagon levels were reported after 4 weeks of treatment in diabetic subjects with LAF-237 [2]. Reduced glucagon concentrations would lower hepatic glucose output during the

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