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Peptide degradation and the role of DPP-4 inhibitors in the treatment of type 2 diabetes

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ABSTRACT

Dipeptidyl peptidase-4 (DPP-4) inhibitors are now a widely used, safe and efficacious class of antidiabetic drugs, which were developed prospectively using a rational drug design approach based on a thorough understanding of the endocrinology and degradation of glucagon-like peptide-1 (GLP-1). GLP-1 is an intestinal hormone with potent insulinotropic and glucagonostatic effects and can normalise blood glucose levels in patients with type 2 diabetes, but the native peptide is not therapeutically useful because of its inherent metabolic instability. Using the GLP-1/DPP-4 system and type 2 diabetes as an example, this review summarises how knowledge of a peptide's biological effects coupled with an understanding of the pathways involved in its metabolic clearance can be exploited in a rational, step-by-step manner to develop a therapeutic agent, which is effective and well tolerated, and any side effects are minor and largely predictable. Other peptides with metabolic effects which can also be degraded by DPP-4 will be reviewed, and their potential role as additional mediators of the effects of DPP-4 inhibitors will be assessed.

1. Introduction

The story leading to the development of inhibitors of the enzyme dipeptidyl peptidase-4 (DPP-4) as a successful therapy for type 2 diabetes (T2DM) could almost be described as a textbook example of how an understanding of basic physiology/endocrinology can be exploited to lead to rational prospective drug design based on a prior knowledge of both the target and the underlying mechanism of action. In this respect, it contrasts to the development of some other classes of oral anti-hyperglycaemic agents, such as the biguanides, sulphonlylureas and thiazolidinediones, whose glucose-lowering activities were discovered before it was understood how they worked. In the example of the DPP-4 inhibitors, the finding that the incretin hormone, glucagon-like peptide-1 (GLP-1) was particularly susceptible to degradation by DPP-4 prompted the suggestion that targeting this route of metabolism may be a novel therapeutic strategy for managing T2DM [1], spurring research into the role of DPP-4 in GLP-1 biology and glucose metabolism and ultimately ending with the development and launch of a successful medicine, the DPP-4 inhibitor (a.k.a. gliptin) class of anti-hyperglycaemic drugs.

GLP-1 is a gut peptide which was discovered following cloning of the gene encoding the pancreatic hormone, glucagon. As well as the sequence of glucagon itself, the proglucagon gene was found to code for two additional sequences which bore strong homology to glucagon, and the existence of two related peptides, GLP-1 and GLP-2, was predicted [2]. GLP-1 was subsequently identified in intestinal extracts from pigs [3] and rats [4], and found to be highly potent in stimulating insulin [5,6] and inhibiting glucagon secretion [7] from the perfused pancreas. Later, GLP-1 was shown to be released in response to food ingestion [8,9] and to act as an incretin hormone, markedly enhancing meal-stimulated insulin secretion [10]. The incretin effect (i.e. the greater insulin response to oral – as opposed to isoglycaemic intravenous infusion of – glucose) was known to be impaired in T2DM [11], so it was of particular interest to find that GLP-1 was insulinotropic in patients with T2DM, and could normalise fasting glucose levels when given intravenously [12]. This raised interest in the possibility of using GLP-1 therapeutically. It came as some disappointment then, when the effects of a single subcutaneous injection of GLP-1 had only a short-lived effect on insulin secretion and failed to bring fasting glucose levels back into the normal range [13]. Nevertheless, repeated subcutaneous injections were effective [13], and when given by continuous subcutaneous infusion, continuous exposure to GLP-1 over 6 weeks improved glycaemic control and caused a small drop in body weight in subjects with T2DM [14], establishing GLP-1 as a viable drug target.

Abbreviations: DPP-4, dipeptidyl peptidase-4; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GRP, gastrin-releasing peptide; NEP, neutral endopeptidase; OXM, oxyntomodulin; PACAP, pituitary adenylate cyclase-activating polypeptide; PYY, peptide tyrosine tyrosine; SDF-1α, stromal cell-derived factor-1α; T2DM, type 2 diabetes mellitus

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At the time, the transient nature of the effect of a single subcutaneous injection of GLP-1 on blood glucose was entirely unexpected. Plasma levels of GLP-1 seemed to be raised significantly [15], and attained levels well above those which were associated with a glucose-lowering effect during an intravenous infusion of GLP-1 [12]. There therefore appeared to be nothing to suggest that the peptide was not stable after the subcutaneous injection, and the reason for the short-lasting effect of GLP-1 remained unexplained.

2. The role of DPP-4 in incretin hormone metabolism

The first hint that GLP-1 might not be completely stable came from a meeting abstract in 1992, reporting that GLP-1 could be degraded in plasma incubations [16], although the enzyme(s) involved was not identified. A year later, Mentlein and colleagues [17], in a search for peptide substrates of the serine protease DPP-4, used high performance liquid chromatography to demonstrate that pharmacological (μmolar) concentrations GLP-1, and the other incretin hormone glucose-dependent insulinotropic polypeptide (GIP), were degraded *in vitro* by the purified enzyme and in plasma to form an N-terminally truncated metabolite, and that this degradation was prevented in the presence of a DPP-4 inhibitor. Although the degradation of physiological peptide concentrations or endogenous peptides were not examined, given that the N-terminus of members of the glucagon/VIP peptide family, to which GLP-1 and GIP belong, is important for receptor activation [18], the authors speculated that DPP-4 action could lead to the loss of biological activity as well as potentially interfere with their measurement by immunoassays [17]. Subsequently, more physiologically relevant (picomolar) levels of GLP-1 were also shown to be degraded by DPP-4 in plasma, and the N-terminally truncated metabolite generated by DPP-4 action was identified as the major circulating form of endogenous GLP-1 in humans, suggesting that DPP-4 was likely to play a physiological role in the metabolism of the peptide *in vivo* [19]. This suggestion was supported by the finding that exogenously administered radio-labelled GLP-1 and GIP were degraded in wild-type, but not in DPP-4 deficient rats by Kieffer et al., [20] who, like Mentlein and colleagues [17], also concluded that DPP-4-mediated degradation of the incretin hormones could interfere with their accurate measurement whilst also being responsible for their inactivation. Conventional immunoassays of the time mostly employed antisera directed towards epitopes in the mid-region or C-terminal end of GLP-1; such assays are insensitive to modifications at the N-terminus of the peptide, meaning that they will also react with any peptides which are either N-terminally elongated or truncated. Therefore, in order to assess the relevance of DPP-4-mediated degradation of GLP-1 in humans, we developed a novel immunoassay for the intact N-terminus of GLP-1. By comparing GLP-1 concentrations measured with this assay against those determined with the conventional type of assay, we showed that exogenously administered GLP-1 underwent substantial degradation in both healthy individuals and subjects with T2DM; after subcutaneous injection, less than 10% of the peptide survived in the intact state, thereby verifying that the commonly used conventional GLP-1 assays considerably overestimated concentrations of the biologically active peptide (because the inactive metabolite was also detected) [1]. Moreover, this thereby provided an explanation for the short-lived effects of GLP-1 in the earlier clinical studies (because the intact biologically active peptide was rapidly degraded by DPP-4), and led to the initial proposal that inhibiting the activity of DPP-4 to reduce this degradation and enhance levels of active GLP-1 may be a novel therapeutic approach to manage T2DM [1]. For this approach to be successful, the DPP-4 enzyme would need to play a key role in the metabolism of GLP-1. From *in vitro* kinetic studies, Mentlein et al. [17] had already predicted that GLP-1 was a particularly good substrate for the enzyme, and *in vivo* studies in rats [20] and humans [1,19] had indicated that the N-terminally truncated metabolite arising from DPP-4-mediated cleavage seemed to be the predominant endogenous [19] and exogenous [1,20] metabolite, and was formed rapidly after exogenous administration of the peptide [1,20]. Endogenous GLP-1 is also exquisitely susceptible to DPP-4-mediated degradation. Although the peptide is stored predominantly in its intact form, over half of the GLP-1 which is secreted from the perfused intestine was found to be already degraded to the N-terminally truncated metabolite by the time it had reached the local blood vessels draining the preparation, and it was concluded that DPP-4, which was shown to be present on the vascular endothelium, including capilliaries adjacent to the GLP-1-producing L-cells, was responsible [21]. Together, these observations suggested that DPP-4 was likely to be the initial and primary route of degradation of GLP-1. Similar conclusions were reached regarding the role of DPP-4 in GIP metabolism. Accordingly, observations from *in vitro* studies [17] and from *in vivo* administration of radio-labelled GIP in rats [20] showing that GIP was degraded by DPP-4 were followed by the development of an N-terminally directed immunoassay to reveal that the metabolite generated by DPP-4 cleavage was the major circulating form of endogenous GIP in humans [22].

Further indirect evidence supporting an important role of DPP-4 in the modulating the actions of both incretins comes from the observations that analogues of GLP-1 and GIP, modified to be resistant to DPP-4 cleavage, were subsequently shown to have greater metabolic stability *in vivo* which was associated with more potent and longer-lasting effects [23–27]. This line of research culminated in the clinical development of stable, long-lasting GLP-1 receptor agonists for the treatment of T2DM.

3. Harnessing the therapeutic potential of DPP-4 inhibition

By the late-1990s, the significance of DPP-4 for the initial degradation of the incretin hormones was becoming more apparent. This had led to the idea that blocking this route of metabolism may elevate levels of the intact biologically active peptides, which should therefore enhance their insulinotropic and hence, glucose-lowering effects, giving the rationale for using DPP-4 inhibition as a means of controlling hyperglycaemia in patients with T2DM [1]. While, theoretically, this was expected to be the case, it was unknown whether, once DPP-4 action was blocked, the half-life of the intact peptide would be prolonged *in vivo* and actually result in greater insulinotropic and glucose-lowering effects or whether any other routes of metabolism would be uncovered, meaning that *in vivo* efficacy would not be not improved.

That DPP-4 was the major, if not the only route of metabolism of GLP-1 *in vivo*, at least acutely, was confirmed when it was shown that a DPP-4 inhibitor (valine-pyrrolidide) could not only completely prevent the degradation of exogenous GLP-1 in anaesthetised pigs, but was also associated with potentiated insulinotropic and antihyperglycaemic effects following an intravenous glucose load [28]. Similar results were subsequently shown for GIP [29]. In rats, administration of a single dose of another DPP-4 inhibitor (ile-thiazolidide) improved insulin secretion and reduced glucose excursions after an oral glucose tolerance test, which was thought to be due to increased circulating levels of intact endogenous incretins [30]. This speculation was subsequently shown to be correct by the demonstration that valine-pyrrolidide fully protected endogenous GLP-1, released from the perfused porcine intestine, from degradation [21], while acute DPP-4 inhibition *in vivo* in rodent models of glucose intolerance using ile-thiazolodide [31], NVP-DPP728 [32] or valine-pyrrolidide [33] inhibited plasma DPP-4 activity and was associated with enhanced glucose-stimulated intact GLP-1 levels and improved glucose tolerance. Together, these studies established pre-clinical proof-of hypothesis that inhibiting DPP-4 activity could potentiate circulating levels of intact GLP-1 to enhance its effects on the endocrine pancreas and improve glucose homeostasis, and were soon followed by studies showing that these beneficial effects were still evident with chronic dosing. Thus, improvements in glucose tolerance were sustained over 12 weeks dosing with ile-thiazolidide in diabetic rats [34], while similar results were obtained in glucose-intolerant insulin-resistant mice treated with NVP-DPP728 for 8 weeks [35].