Diabetologia (2005) 48: 608–611
DOI 10.1007/s00125-005-1704-8

FOR DEBATE

M. A. Nauck · A. El-Ouaghlidi

The therapeutic actions of DPP-IV inhibition are not mediated by glucagon-like peptide-1

Received: 30 November 2004 / Accepted: 6 January 2005 / Published online: 11 March 2005

Keywords Dipeptidyl peptidase-IV · Gastric inhibitory polypeptide · Gastroenteropancreatic peptide hormones · Glucagon-like peptide-1 · Incretin · Incretin effect · Oral hypoglycaemic agents · Pharmacokinetics · Proteolytic processing

Abbreviations GIP: gastric inhibitory peptide · GLP-1: glucagon-like peptide-1 · DPP-IV: dipeptidyl peptidase-IV

Introduction

Glucagon-like peptide 1 (GLP-1) and its derivatives have generated considerable interest [1]. GLP-1 has the potential to normalise glucose concentrations in patients with type 2 diabetes; however, this peptide hormone is degraded and eliminated from the circulation too rapidly to be of therapeutic value. The initial step of proteolytic cleavage and inactivation is mediated by dipeptidyl peptidase-IV (DPP-IV), following which, only 10–15% of the peptide remains in its intact, biologically active form during the continuous infusion of GLP-1 [2]. DPP-IV inhibitors reverse this effect, increasing circulating levels by a factor of 4–6 during the infusion of exogenous GLP-1 [3], thus providing the rationale for their use in diabetes therapy. Phase II studies have shown that DPP-IV inhibitors can lower HbA_1c levels by approximately 1%, thus confirming their therapeutic potential [4].

DPP-IV inhibitors have been developed to augment circulating concentrations of intact, biologically active, endogenously secreted GLP-1, and it is widely believed that the effects of DPP-IV inhibition are largely mediated by GLP-1. Gastric inhibitory peptide (GIP) is also a substrate of DPP-IV, but its insulinotropic effect is reduced in patients with, or at risk of, type 2 diabetes [5]. Other incretin candidates have not been convincingly characterised, and DPP-IV inhibitors are ineffective in mice in which both the GLP-1 and GIP receptors have been knocked out [6]. These observations would seem to point to GLP-1 as the sole (or major) mediator of the therapeutic effect of DPP-IV inhibition; however, there are other arguments that oppose this view.

M. A. Nauck (✉) · A. El-Ouaghlidi
Diabeteszentrum Bad Lauterberg,
Kirchberg 21,
37431 Bad Lauterberg im Harz, Germany
e-mail: M.Nauck@diabeteszentrum.de
Tel.: +49-5524-81318
Fax: +49-5524-81298

DDP-IV inhibition causes little increase in endogenous GLP-1

Although DPP-IV inhibition results in a fourfold to sixfold increase in circulating levels of active GLP-1 following infusion of the peptide, no comparable effect upon endogenous GLP-1 secretion has been observed (Table 1, Fig. 1a). In contrast, the majority of studies have failed to demonstrate an at least 100% increase in the concentration of intact biologically active GLP-1 [7–12], and the infusion of an equivalent amount of GLP-1 (e.g. 0.2–0.4 $pmol \cdot kg^{-1} \cdot min^{-1}$) has little insulinotropic activity in healthy subjects and even less activity in patients with type 2 diabetes [13]. In the latter study, physiological replacement doses of GLP-1 did not produce significant increases in insulin secretion under hyperglycaemic clamp conditions in patients with type 2 diabetes [13]. Other studies have indeed found effects of relatively low doses of exogenous GLP-1 in type 2 diabetes [14], but on closer analysis of their dose–response curves for insulinotropic effects it is not clear that minor elevations in plasma GLP-1 (as demonstrated with DPP-4 inhibition; Table 1, Fig. 1) would produce a clinically meaningful insulinotropic response.

DPP-IV inhibitors have little effect on gastric emptying

GLP-1 slows gastric emptying [15], and dose–response curves indicate that even the lowest concentrations of (active) GLP-1 associated with insulinotropic [15] and glucose-lowering [16] effects lead to the significant decel-

Table 1 Effects of DPP-IV inhibitors on plasma concentrations of intact, biologically active GLP-1 and total (i.e. including metabolites) GLP-1

Study	Species/ condition	DPP-IV inhibitor	Dose	Duration of treatment	Plasma active GLP-1 (pmol/l)				Plasma total GLP-1 (pmol/l)				Comments
					Placebo		DPP-IV inhibitor		Placebo		DPP-IV inhibitor		
					Basal	Peak	Basal	Peak	Basal	Peak	Basal	Peak	
Balkan et al. [7]	*fa/fa* Zucker rats	NVP-DPP728	10 μmol/kg orally	Single dose	0[a]	≈8	0[a]	≈30	0[a]	≈16	0[a]	≈32	No feedback inhibition; 100% active GLP-1with DPP-IV inhibition
Pauly et al. [8]	Wistar rats	Ile thiazolidide	1.5 μmol/0.75 μmol per min i.v.	30 min	–[b]	51±4	–[b]	85±6	Not determined				Increment in intact GLP-1 with DPP-IV inhibition
Sudre et al. [26]	*fa/fa* Zucker rats	FE999011	3 mg/g i.v.	Single dose	≈2	≈2	≈2	≈10	Not determined				No increment in active GLP-1 without DPP-IV inhibition
Reimer et al. [9]	Mice	NVP-DPP728	0.12 μmol/g		0[a]	4±2[c]	0[a]	17±3[c]	Not determined				Clear increment in active GLP-1 with DPP-IV inhibition
					0[a]	5±2[d]	0[a]	12±2[d]	Not determined				
Deacon et al. [22]	Female beagles	NVP-DPP728	1 mg/kg		≈6	≈13	≈25	≈45	≈20	≈65	≈30	≈47	Reduction in total, but clear increment in active GLP-1; similar results for GIP
Larsen et al. [10]	Diabetic minipigs[e]	Valine pyrrolidide	50 mg/kg		≈18	≈21	≈12	≈35	≈10	≈29	≈15	≈26	No significant effect of DPP-IV inhibition on intact GLP-1, but effect on GIP
Dardik et al. [11]	Cynomolgus monkeys	LAF237	1 μmol/kg	Single dose	0[a]	4±3	0[a]	20±7	Not determined				Gastric emptying retarded, but late after oral glucose.
Åhren et al. [12]	Type 2 diabetic patients	LAF237	100 mg/day	4 weeks	4±1	8±1	6±1	17±2	Not determined				DPP-IV inhibition doubles active GLP-1 levels; gastric emptying not measured
El-Ouaghlidi et al. [23]	Healthy human subjects	LAF237	100 mg	Single dose	Not determined				10±2	26±5	10±2	14±2	Significant reduction in GLP-1 secretion (total GLP-1): feedback inhibition? Gastric emptying not changed
Herman et al. [27]	Type 2 diabetic patients	MK-0431	25 mg/200 mg	Single dose	–	–[f]	–	–[f]					DPP-IV inhibition (both doses) doubles intact GLP-1 and ratio of active/total GLP-1; gastric emptying not measured

Values are means±SEM or approximate mean values (marked with ≈)
[a]Changes in GLP-1 concentrations (increments over basal) reported
[b]Values were below the detection limit of the method
[c]Experiments with a normal diet
[d]Experiments with a high-fat diet
[e]Diabetes induced by nicotinamide/streptozotocin
[f]Peak with DPP-IV inhibition reported to be approximately doubled

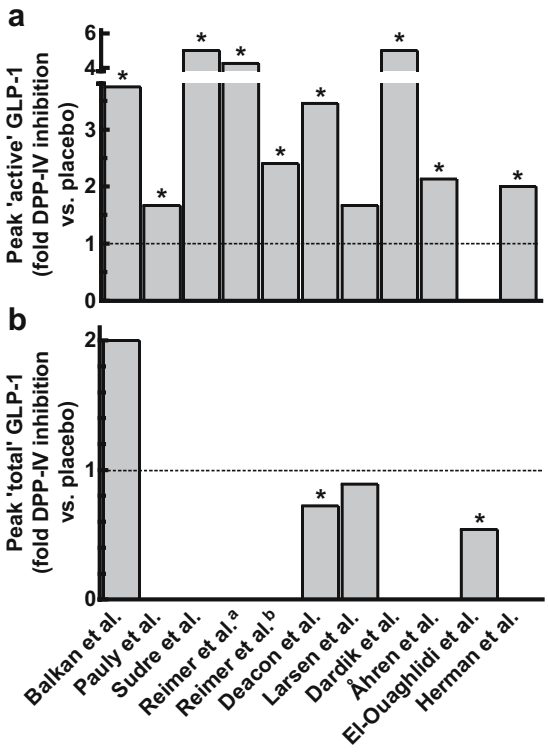

Fig. 1 GLP-1 concentrations after the oral intake of nutrients expressed as the peak values after DPP-IV inhibition relative to placebo. **a** Intact, biologically active GLP-1, as determined with an N-terminal-specific antiserum. **b** Total GLP-1 including metabolites generated by DPP-IV action, as determined with a non-specific immunoassay, and indicating the total amount of DPP-IV secreted. Details of the references are provided in Table 1. All studies reported significantly higher concentrations of active GLP-1 with DPP-IV inhibition. Some, but not all studies, demonstrated a significant reduction in total GLP-1 with DPP-IV inhibition. [a]Studies with normal diets. [b]Studies with high-fat diets. *Peak GLP-1 concentrations (or integrated incremental values) significantly different vs placebo

eration of gastric emptying. This is also true for other incretin mimetics, such as exenatide [17] and liraglutide [18]. It should be noted that a decrease in the rate of gastric emptying has not been observed in clinical studies on DPP-IV inhibitors [19]. Furthermore, the studies published to date do not provide indirect evidence (e.g. delayed increments in glucose and parameters of insulin secretion following nutrient intake) of delayed gastric emptying [4]. The single exception to this is a study in monkeys, but in this case the differences in gastric emptying occurred later than the increase in intact, biologically active GLP-1 [11]. It may therefore be considered unlikely that DPP-IV inhibition leads to plasma concentrations that are high enough to delay gastric emptying and, hence, stimulate insulin secretion.

GLP-1 and incretin mimetics cause nausea/vomiting while DPP-IV inhibitors do not

In clinical studies, incretin mimetics such as exenatide [20] and liraglutide [21] invariably cause nausea and vomiting in a proportion of patients. This adverse effect is less marked with GLP-1 [1], but the absence of such side effects in studies with DPP-IV inhibitors can be interpreted as evidence against the presence of high ('therapeutic') levels of endogenously secreted intact GLP-1.

Meal-stimulated levels of GLP-1 fall in response to DPP-IV inhibition

Meal or oral glucose-stimulated GLP-1 secretion is reduced, even following a single oral dose of a DPP-IV inhibitor (Fig. 1b), in both dogs [22] and healthy humans [23]. The reported reduction in humans was 75%, as measured in terms of integrated incremental concentrations of total GLP-1 after an oral glucose load, using a non-specific radioimmunoassay that is not sensitive to the presence or absence of an intact N-terminus. This implies that the maximum possible circulating concentration of intact, biologically active GLP-1 is around 25% of that seen in placebo-treated subjects, leaving little potential for plasma concentrations of endogenously secreted GLP-1 to rise into the therapeutically relevant range. Furthermore, the longer-term effects of the repeated administration of DPP-IV inhibitors upon post-meal increments in intact GLP-1 are unknown. However, it appears certain that the reduction in GLP-1 secretion produced by DPP-IV inhibition is not compensated for by the hypersecretion of other incretin hormones, since GIP secretion is significantly reduced after treatment with LAF237 [23]. This finding is supported by a study showing smaller increments in intact GLP-1 and intact GIP in rats lacking DPP-IV [24].

DPP-IV inhibition has delayed effects on glucose homeostasis

GLP-1 has glucose-lowering effects, both during and after its administration [1], as do incretin mimetics such as exenatide and liraglutide [17, 18]. Based on the assumption that the effects of DPP-IV inhibition are mediated by GLP-1, one might expect that the maximum clinical effectiveness of inhibition would be reached within a few hours. This is not the case, and clinical studies have shown that reductions in fasting glucose and other measures of glycaemic control improve much more slowly, reaching maximum values after weeks or even months [4].

Conclusion

Taken together, these arguments cast doubt on the assumption that GLP-1 is the only, or at least the major, mediator of the clinical effects of DPP-IV inhibition. Therefore, more effort should be put into elucidating the role of other potential incretin hormones or neuropeptides, such as pituitary adenylate cyclase-activating polypeptide [25]. The importance of neuropeptides as mediators may have been underestimated due to the fact that their effect cannot be

related to measurable plasma concentrations. Regardless of this, it may be assumed that better knowledge of the mechanisms involved in the therapeutic effects of DPP-4 inhibition will allow these agents to be used more effectively to meet the needs of patients with type 2 diabetes.

Acknowledgements The authors' work has been supported by grants from the German Research Foundation (grant no. Na 203/6-1) and Novartis Pharma (Basel, Switzerland). We thank S. Petrick for help with literature search and the preparation of the figures.

References

1. Nauck MA, Meier JJ, Creutzfeldt W (2003) Incretins and their analogues as new antidiabetic agents. Drug News Perspect 16:413–422
2. Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Host JJ (1995) Both subcutaneously and intravenously administered glucagon-like peptide 1 are rapidly degraded from the NH_2-terminus in type 2-diabetic patients and in healthy subjects. Diabetes 44:1126–1131
3. Deacon CF, Hughes TE, Holst JJ (1998) Dipeptidyl peptidase IV inhibition potentiates the insulinotropic effect of glucagon-like peptide 1 in the anesthetized pig. Diabetes 47:764–769
4. Åhren B, Gomis R, Standl E, Mills D, Schweizer A (2004) Twelve- and 52-week efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin-treated patients with type 2 diabetes. Diabetes Care 27:2874–2880
5. Nauck MA, Baller B, Meier JJ (2004) Gastric inhibitory polypeptide and glucagon-like peptide-1 in the pathogenesis of type 2 diabetes. Diabetes 53[Suppl 3]:S190–S196
6. Hansotia T, Baggio LL, Delmeire D et al (2004) Double incretin receptor knockout (DIRKO) mice reveal an essential role for the enteroinsular axis in transducing the glucoregulatory actions of DPP-IV inhibitors. Diabetes 53:1326–1335
7. Balkan B, Kwasnik L, Miserendino R, Holst JJ, Li X (1999) Inhibition of dipeptidyl peptidase IV with NVP-DPP728 increases plasma GLP-1 (7–36 amide) concentrations and improves oral glucose tolerance in obese Zucker rats. Diabetologia 42:1324–1331
8. Pauly RP, Demuth HU, Rosche F et al (1999) Improved glucose tolerance in rats treated with the dipeptidyl peptidase IV (CD26) inhibitor Ile-thiazolidide. Metabolism 48:385–389
9. Reimer MK, Holst JJ, Åhren B (2002) Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. Eur J Endocrinol 146:717–727
10. Larsen MO, Rolin B, Ribel U et al (2003) Valine pyrrolidide preserves intact glucose-dependent insulinotropic peptide and improves abnormal glucose tolerance in minipigs with reduced beta-cell mass. Exp Diabetes Res 4:93–105
11. Dardik B, Valentin M, Schwartzkopf C et al (2003) NVP-LAF237, a dipeptidyl peptidase IV inhibitor, improves glucose tolerance and delays gastric emptying in obese insulin resistant cynomolgus monkeys (abstract 1292-P). Diabetes 52[Suppl 1]: A322
12. Åhren B, Gomis R, Mills D, Schweizer A (2004) The DPP-IV inhibitor, LAF237, improves glycemic control in patients with Type 2 diabetes (T2DM) inadequately treated with metformin (abstract 354-OR). Diabetes 53[Suppl 2]:A83
13. Nauck MA, Heimesaat MM, Ørskov C, Holst JJ, Ebert R, Creutzfeldt W (1993) Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest 91:301–307
14. Kjems LL, Holst JJ, Vølund A, Madsbad S (2003) The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects. Diabetes 52:380–386
15. Nauck MA, Niedereichholz U, Ettler R et al (1997) Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am J Physiol Endocrinol Metab 273:E981–E988
16. Meier JJ, Gallwitz B, Salmen S et al (2003) Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. J Clin Endocrinol Metab 88:2719–2725
17. Kolterman OG, Buse JB, Fineman MS et al (2003) Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. J Clin Endocrinol Metab 88:3082–3089
18. Juhl CB, Hollingdal M, Sturis J et al (2002) Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. Diabetes 51:424–429
19. El-Ouaghlidi A, Rehring E, Schweizer A, Holmes D, Nauck MA (2003) The dipeptidyl peptidase IV inhibitor LAF237 does not accentuate reactive hypoglyaemia caused by the sulfonylurea glibenclamide administered before an oral glucose load in healthy subjects (abstract 507-P). Diabetes 52[Suppl 1]:A118
20. Buse JB, Henry RR, Han J, Kim DD, Fineman MS, Baron AD (2004) Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. Diabetes Care 27:2628–2635
21. Madsbad S, Schmitz O, Ranstam J, Jakobsen G, Matthews DR (2004) Improved glycemic control with no weight increase in patients with type 2 diabetes after once-daily treatment with the long-acting glucagon-like peptide 1 analog liraglutide (NN2211): a 12-week, double-blind, randomized, controlled trial. Diabetes Care 27:1335–1342
22. Deacon CF, Wamberg S, Bie P, Hughes TE, Holst JJ (2002) Preservation of active incretin hormones by inhibition of dipeptidyl peptidase IV suppresses meal-induced incretin secretion in dogs. J Endocrinol 172:355–362
23. El-Ouaghlidi A, Rehring E, Holst JJ, Schweizer A, Holmes D, Nauck MA (2004) Reduced increments in total glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) in plasma after a single dose of the dipeptidyl peptidase-4 inhibitor LAF237 before an oral glucose load in healthy subjects (abstract 786). Diabetologia 47[Suppl 1]:A284
24. Pederson RA, Kieffer TJ, Pauly R, Kofod H, Kwong J, McIntosh CH (1996) The enteroinsular axis in dipeptidyl peptidase IV-negative rats. Metabolism 45:1335–1341
25. Sherwood NM, Krueckl SL, McRory JE (2000) The origin and function of the pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon superfamily. Endocr Rev 21:619–670
26. Sudre B, Broqua P, White RB et al (2002) Chronic inhibition of circulating dipeptidyl peptidase IV by FE 999011 delays the occurrence of diabetes in male Zucker diabetic fatty rats. Diabetes 51:1461–1469
27. Herman GA, Zhao P-L, Dietrich B et al (2004) DP-IV inhibitor MK-0431 enhances active GLP-1 and reduces glucose following an OGTT in type 2 diabetics (abstract 353-OR). Diabetes 53 [Suppl 2]:A82