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1

Incretin hormone mimetics and analogues in diabetes therapeutics

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The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are physiological gut peptides with insulin-releasing and extrapancreatic glucoregulatory actions. Incretin analogues/mimetics activate GLP-1 or GIP receptors whilst avoiding physiological inactivation by dipeptidyl peptidase 4 (DPP-4), and they represent one of the newest classes of antidiabetic drug. The first clinically approved GLP-1 mimetic for the treatment of type-2 diabetes is exenatide (Byetta/exendin) which is administered subcutaneously twice daily. Clinical trials of liraglutide, a GLP-1 analogue suitable for once-daily administration, are ongoing. A number of other incretin molecules are at earlier stages of development. This review discusses the various attributes of GLP-1 and GIP for diabetes treatment and summarises current clinical data. Additionally, it explores the therapeutic possibilities offered by preclinical agents, such as non-peptide GLP-1 mimetics, GLP-1/glucagon hybrid peptides, and specific GIP receptor antagonists.

Key words: incretin; glucagon-like peptide-1; glucose-dependent insulinotropic polypeptide; insulin; type-2 diabetes; glucose homeostasis; exendin.

INCRETINS AND THE ENTEROINSULAR AXIS

The enteroinsular axis was first described in the 1960s following experiments demonstrating that a greater insulin secretory response could be elicited by oral as opposed to intravenous administration of glucose.[1,2] The enteroinsular axis comprises nutrient,

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neural and incretin hormone signals that activate pancreatic β cells.[3] Incretin hormones are peptides which are released in response to nutrients and which stimulate insulin secretion at physiological concentrations.[4] The main incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are released from intestinal endocrine L and K cells, respectively. GLP-1 is produced by post-translational processing of preproglucagon gene products, and the predominant form in human plasma is GLP-1(7–36)amide.[5] GIP is secreted as a 42-amino-acid peptide and shares approximately 68% sequence similarity with GLP-1.[6] The physiological responses to GLP-1 and GIP secreted in response to feeding are elicited by the binding and activation of separate transmembrane G-protein-coupled receptors that have no cross-reactivity.[6] Receptors for GLP-1 and GIP are found in many organs, including pancreas, stomach, skeletal muscle, heart, lung and brain. Comprehensive up-to-date details on the biology of both incretin hormones can be found elsewhere.[7]

Diabetes is rapidly becoming a global health epidemic, with recent projections indicating that over 366 million people will be affected by 2030.[8] Type-2 diabetes constitutes more than 90% of these cases, and is characterized by β-cell decline, insulin resistance, and increased hepatic glucose production. This form of diabetes is also associated with a defect in the endogenous incretin system.[9] Thus, the contribution of incretin factors to the total insulin response to enteral stimulation is decreased in diabetic patients compared with control subjects.[9] This reflects a modest but significant decrease in meal-stimulated GLP-1[10] and disturbed GIP amplification of the late phase of glucose-regulated insulin release.[11,12]

ANTIDIABETIC EFFECTS OF THE INCRETIN HORMONES

As summarised in Table 1, GLP-1 and GIP possess a number of antidiabetic actions.[7] An array of effects across a range of physiological systems enhances blood glucose

Table 1. Comparison of the antidiabetic actions of the incretin hormones.

Physiological effect	Incretin hormone	
	GLP-1	GIP
Lower blood glucose	Yes	Yes
Glucose-dependent stimulation of insulin secretion	Yes	Yes
Enhance β-cell glucose responsiveness	Yes	Yes
Increase β-cell gene expression and differentiation	Yes	Yes
Suppress glucagon secretion	Yes	No
Stimulate somatostatin secretion	Yes	No
Stimulate β-cell expansion	Yes	Yes
Enhance β-cell survival	Yes	Yes
Extrapancreatic glucose-lowering actions	Yes	Yes
Suppress gastric acid secretion	No	Yes
Inhibition of gastric emptying	Yes	No
Inhibition of hepatic insulin extraction	Yes	Yes
Enhance satiety	Yes	No
Reduce body weight	Yes	No

GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic polypeptide.

control, but also provides exploitable opportunities to potentially preserve β-cell function and slow the progression of type-2 diabetes.

Of key importance are the potent insulinotropic activities of GLP-1 and GIP which are triggered only when blood glucose levels become elevated. This glucose-dependent mode of action is not offered by other insulin-releasing drugs, such as the sulphonylureas or meglitinides, and therefore provides a key advantage in minimising episodes of hypoglycaemia.[13,14] Similarly, the incretin hormones promote insulin biosynthesis and improve β-cell responsiveness to glucose by enhancement of functional gene expression. GLP-1 also inhibits secretion of glucagon from pancreatic α cells[15], possibly mediated through increased somatostatin secretion.

Besides these actions on the endocrine pancreas, blood glucose control is further enabled by several extrapancreatic actions of the incretin hormones. There is evidence that GLP-1 and GIP have 'insulin-like effects' in peripheral tissues, enhancing glucose uptake in liver, adipose and muscle tissues.[16–20] Also, in human subjects, GLP-1 reduces hepatic glucose production, and both hormones may reduce hepatic insulin extraction.[21–23] GIP inhibits gastric acid secretion[24] and, more importantly, GLP-1 inhibits gastric emptying[25,26], thereby slowing the digestion and absorption of carbohydrates. This effect on gastric emptying is considered to be a key instrument in the antidiabetic action of GLP-1.[27]

There is compelling evidence that the clinical use of GLP-1 analogues/mimetics promotes significant weight loss.[28–32] Mechanisms through which GLP-1 induces weight loss include the stimulation of feelings of satiety, inhibition of gastric emptying, and possibly increased energy expenditure.[25,26,33,34] Given the strong correlation between type-2 diabetes and obesity, and the fact that weight loss improves insulin sensitivity and delays progression of diabetes, this attribute offers a significant advantage over many other antidiabetic drugs, such as sulphonylureas or thiazolidenediones, which tend to promote weight gain.

Several studies indicate that GLP-1 and GIP have protective and proliferative effects on the pancreatic β cell.[35–41] Recent studies show that GLP-1 counteracts endoplasmic reticulum stress in the β cell[42] and prevents cytokine-induced cell death by inhibiting JAK1-STAT1.[43] Furthermore, a stable GIP analogue enhances functional differentiation of mouse embryonic stem cells into cells expressing islet-specific genes and hormones.[44] Although clinical studies to confirm such effects are not yet available, there are indications that native GLP-1 improves β- and α-cell function following intrahepatic islet transplantation in type-1 diabetic patients.[45]

DIPEPTIDYL PEPTIDASE 4 (DPP-4)

Dipeptidyl peptidase 4 (DPP-4) is a complex and widely expressed enzyme which can be found either membrane-anchored or solubilized in blood. The multifaceted roles of DPP-4, such as activation of T lymphocytes and cleavage of numerous physiological peptides, are detailed elsewhere.[46,47] The relevance of DPP-4 to the incretin hormones is that it is the primary physiological inactivator of GLP-1 and GIP, giving half-lives of 2 min and 5–7 min, respectively.[48] As illustrated in Figure 1, such inactivation occurs by the specific cleavage of N-terminal dipeptides from the incretins. Truncation of GLP-1(7–36)amide to GLP-1(9–36)amide reduces receptor affinity 1000-fold and completely eliminates insulin-releasing activity.[49–51] Similar truncation of GIP(1–42) to GIP(3–42) also eliminates insulinotropic activity but reduces receptor affinity only four-fold.[52] The physiological inactivation of GLP-1 and GIP by DPP-4 has

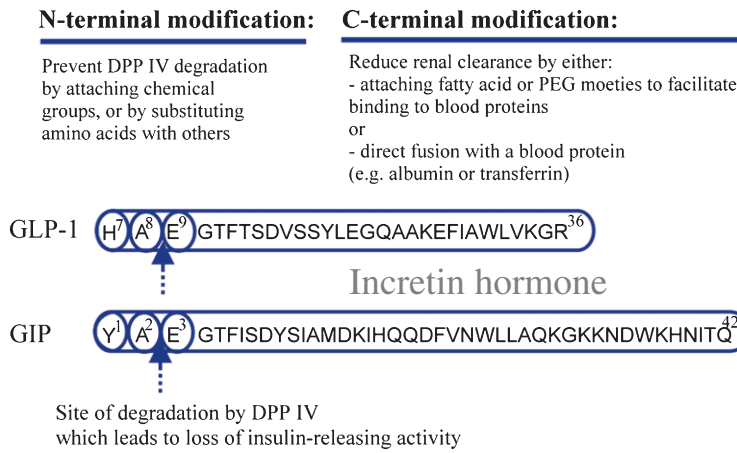

Figure 1. Incretin hormone modification strategies. The incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) can be modified either N-terminally to prevent DPP-4 degradation, or C-terminally to circumvent renal filtration.

been a major obstacle to their clinical implementation and is being overcome by two new pharmacological strategies. The first, which is the main focus of this review, is the development of incretin analogues/mimetics that are resistant to DPP-4. The second, which is dealt with in detail elsewhere in this issue (Chapter 3 by Ahren), is the development of selective inhibitors of DPP-4 which exploit the natural antidiabetic effects of endogenously secreted GLP-1 and GIP.[47,53,54]

PRECLINICAL DEVELOPMENT OF INCRETIN ANALOGUES

N-terminal modification of GLP-1 and GIP to confer resistance to DPP-4

Certain modifications/substitutions of amino acids at the N-terminus of GLP-1 or GIP generate DPP-4-resistant analogues (Table 2). Published information regarding the in vitro and in vivo characteristics of these N-terminally-modified GLP-1 and GIP analogues is detailed in a recent review.[55] Most modifications convey DPP-4 resistance, but receptor activation and biological activity can vary significantly. For GLP-1, Ala^8 substitutions appear to be superior to changes at His^7, whereas Tyr^1 modifications provide more potent GIP agonists than substitutions at Ala^2. In the case of GIP, super-agonists can be generated, as exemplified by N-acetyl GIP.[56] In contrast, amino acid substitutions in either incretin adjacent to the DPP-4 cleavage site at Glu^9 of GLP-1 or Glu^3 of GIP provide weak agonists or even receptor antagonists.[51,57,58]

Two examples of DPP-4-resistant incretin analogues with significant antidiabetic activity in preclinical studies are (Val^8)GLP-1 and N-acetyl GIP. The in vivo effects of (Val^8)GLP-1 are both greater and longer lasting than those of native GLP-1.[37] Once-daily administration of (Val^8)GLP-1 for 3 weeks in *ob*/*ob* mice reduced plasma glucose, increased insulin and decreased body weight substantially more than native GLP-1.[37] Treatment with (Val^8)GLP-1 also improved glucose tolerance, reduced the glycaemic excursion after feeding, increased insulin secretory responsiveness, and improved insulin sensitivity. Morphological studies also indicated that (Val^8)GLP-1 increased islet

Table 2. Analogues of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) bearing N-terminal modification for potential dipeptidylpeptidase 4 (DPP-4) resistance and C-terminal manipulation to counter renal filtration.[55,57–59,63–68,104,105]

GLP-1 analogues	
Modifications at His^7GLP-1	Desamino-GLP-1, (D-His^7)GLP-1, N-glucitol-GLP-1, N-Imi-GLP-1, N-alpha-Me-GLP-1, N-Me-GLP-1, N-acetyl-GLP-1, N-pryoglutamyl-GLP-1
Substitutions at Ala^8GLP-1	(D-Ala^8)GLP-1, (Gly^8)GLP-1, (Ser^8)GLP-1, (Aha^8)GLP-1, (Thr^8)GLP-1, (Aib^8)GLP-1, (Abu^8)GLP-1, (Val^8)GLP-1
Substitutions at Glu^9GLP-1	(Asp^9)GLP-1, (Ala^9)GLP-1, (Pro^9)GLP-1, (Phe^9)GLP-1, (Lys^9)GLP-1, (Tyr^9)GLP-1
C-terminal modifications	Fatty acid acylation, PEGylation, chemical linker (sometimes with enabling Lys substitutions) to promote binding to plasma proteins Direct fusion with albumin or transferrin.
GIP analogues	
Modifications at Tyr^1GIP	N-acetyl-GIP, N-pyroglutamyl-GIP, N-glucitol-GIP, N-palmitate-GIP, N-Fmoc-GIP
Substitutions at Ala^2GIP	(Gly^2)GIP, (Ser^2)GIP, (D-Ala^2)GIP, (Abu^2)GIP, (Sar^2)GIP
Substitutions at Glu^3GIP	(Ala^3)GIP, (Hyp^3)GIP, (Lys^3)GIP, (Phe^3)GIP, (Pro^3)GIP, (Trp^3)GIP, (Tyr^3)GIP
C-terminal modifications	Fatty acid acylation, PEGylation (sometimes with enabling Lys substitutions) to promote binding to plasma proteins

PEG, polyethylene glycol.

area without changing the number of islets.[37] Broadly similar effects, with the exception of body weight changes, were noted in *ob*/*ob* mice receiving once-daily injections of N-acetyl GIP[56,59] This observation also indicates that stable GIP agonists do not suffer from possible desensitization of the GIP receptor as observed in several preclinical animal models.

Although N-terminal modifications can prevent DPP-4 degradation and extend the half-life of incretin peptides, gradual elimination by the kidneys means that biological activity is unlikely to exceed 4 h. As such, these analogues have limited utility for once-daily administration. This is especially true for GLP-1, which is particularly susceptible to DPP-4-mediated degradation, and where renal extraction can be up to 70%. Importantly, the potent GLP-1-receptor agonist, exendin-4 (isolated from saliva of the lizard *Heloderma suspectum*) does not suffer this fate and exerts in-vivo biological effects for up to 7 hours. As a consequence, this GLP-1 mimetic has progressed most rapidly through to clinical application.[60]

Strategies to circumvent renal filtration

A number of approaches has been used to circumvent the rapid renal filtration of incretin hormones (Table 2). The strategies developed either facilitate binding of the incretin hormone to circulating plasma proteins, or involve direct chemical fusion of the incretin hormone to a plasma protein (e.g. albumin or transferrin) prior to administration. Adherence of GLP-1 or GIP to these macromolecules thereby prevents simple filtration in the nephrons of the kidney. This approach has been exploited extensively with a significant number of the GLP-1 peptides, many of which have progressed to various stages of pharmaceutical drug testing (Table 3).

Table 3. Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) in the development of type-2 diabetes therapeutics.

Name	Structure	Status	Company
Exenatide (Byetta)	Exendin-4(1−39) (unmodified) (see also Figure 2)	Approved and launched	Amylin/Eli Lilly
Exenatide LAR	Long-acting release exendin-4(1−39) • polylactide-glycolide microsphere suspension	Phase III	Amylin/Eli Lilly/Alkermes
Liraglutide	GLP-1 plus • C_{16} fatty acid chain linked to Lys^{26} • Lys^{34} replaced with Arg^{34} (see also Figure 2)	Phase III	NovoNordisk
CJC-1131	GLP-1 plus • Gly^{37} replaced with Lys^{37} • Lys37 contains a reactive chemical linker • Ala8 replaced with $D\text{-}Ala^{8}$	Phase II	Conjuchem Inc
CJC-1134-PC	Exendin-4(1−39) with chemical linker as for CJC-1131	Phase II	Conjuchem Inc
ZP 10A	Exendin-4(1−39) plus • N-terminally extended with a His • C-terminally extended with 6 Lys residues	Phase II	Zealand/Aventis
BIM51077	GLP-1 analogue No structural information available	Phase I	Roche/Ipsen
Naliglutide (albugon)	Recombinant GLP-1-albumin protein	Preclinical trials	Human Genome/Glaxo-SmithKline
LY315902	GLP-1 plus • C_8 fatty acid chain linked to Lys^{34} • Lys26 replaced with Arg^{26} • His7 replaced with $des\text{-}His^{7}$	Preclinical trials	Eli Lilly
GLP-1-Tf	GLP-1 fused to the serum protein transferrin (Tf)	Preclinical trials	Biorexis
DAPD (dual-acting peptide for diabetes)	GLP-1/glucagon hybrid peptide	Preclinical trials	Bayer Healthcare Pharmaceuticals
PEG-DAPD (PEGylated DAPD)	GLP-1/glucagon hybrid peptide containing a maleimide-polyethylene glycol polymer	Preclinical trials	Bayer Healthcare Pharmaceuticals
GLP-1-I.N.T.	GLP-1 analogue No structural information available	Preclinical trials	Transition Therapeutics/Novo Nordisk
N-GIP	N-terminally-modified GIP	Preclinical trials	Amylin/Diabetica
CVX-73	DPP IV resistant GLP-1 analogue fused with a proprietory monoclonal antibody	Preclinical trials	CovX Research LLC

Two methods employed to facilitate plasma protein binding involve acylation (attachment of a fatty acid side-chain) or, less frequently, PEGylation (attachment of polyethylene glycol chains). Acylation of GLP-1 with a C_8 fatty acid (LY315902, Eli Lilly; Table 3) results in a half-life of 3–6 h in dogs[61], and C_{16} fatty acid (Liraglutide, NovoNordisk; Table 3) extends half-life to 8 h in humans.[62] Similarly, addition of a C_{16} fatty acid moiety to native, N-acetyl or N-pyroglutamyl GIP generates stable GIP agonists with enhanced antidiabetic activity in preclinical models.[59,63–65] PEGylated forms of GLP-1 and GIP have also been produced and tested.[66–68] The half-life of PEGylated GLP-1 was 40-, 10- and 28-fold longer than that of GLP-1 in plasma, liver and kidney homogenates, respectively.[67] Over a 3-hour period PEGylated GLP-1 improved glucose tolerance in diabetic *db*/*db* mice by up to 63%, compared with only 40% for native GLP-1.[67] PEGylation of GLP-1 at Lys^{34} (as opposed to Lys^{26} or the N-terminus) generates an analogue with the highest biological activity.[68] Dual modification of GIP by N-terminal acylation and C-terminal PEGylation generates a full agonist of comparable potency to native GIP which is stable to DPP-4 cleavage.[66] These molecules have yet to proceed to pharmaceutical development, but remarkably the use of a reactive chemical linker to form covalent peptide bonds with circulating albumin[69,70] extends the half-life of GLP-1 to 18 h (CJC-1131, Conjuchem; Table 3) and exendin-4 to 7 days (CJC-1134, Conjuchem; Table 3). Finally, two novel GLP-1-plasma protein molecules are in early development (Table 3). Albugon (naliglutide; Human Genome/GlaxoSmithKline) is a recombinant GLP-1-albumin protein which is DPP-4-resistant and is expected to have a superior pharmacokinetic profile than native GLP-1.[71] GLP-1 has also been fused to the circulating protein transferrin (GLP-1-Tf; Biorexis), but relatively little information is currently available on the efficacy of either of these latter two modified forms of GLP-1. CVX-73 was created by fusing a DPP IV resistant analogue to a proprietary monoclonal antibody. Preclinical studies in rodents and primates demonstrate efficacy to improve glucose tolerance and protracted duration of action with effects persisting up to 5 days post administration.[107]

Non-peptide incretin mimetics

Incretin analogues/mimetics developed thus far must be delivered subcutaneously because their peptide nature makes them unsuitable for oral administration. The recent surge in development of DPP-4 inhibitors is perhaps because these compounds readily lend themselves to oral administration. However, there have been recent developments in the search for non-peptide GLP-1 agonists.[72,73] A library of 48,160 synthetic and natural compounds was screened for ability to bind to the GLP-1 receptor and stimulate production of adenylate cyclase.[72] After evaluation of five initial hits, a small-molecule candidate compound, Boc5 (a substituted cyclobutane), was identified that stimulated glucose-dependent insulin secretion and lowered food intake in mice. The effects of Boc5 were countered by co-administration of the established GLP-1 receptor antagonist, exendin(9–39). Furthermore, injection of Boc5 to *db*/*db* mice over a 6-week period significantly lowered HbA1c values.[72] In another report, a combination of structural and functional screening assays was used to identify two allosteric modulators selective for the GLP-1 receptor from an initial screen of 500,000 small molecules.[73] The most potent molecule identified (compound 2; a substituted quinoxaline), stimulated glucose-induced insulin release from mouse islets, but in-vivo glucoregulatory activity of the compound was not reported.

Despite these advances, the considerable effort and luck required to develop small non-peptide molecules as therapeutically useful incretin mimetics should not be

underestimated. Key issues for confrontation include synthesis, specificity, toxicity and pharmacodynamics, as well as acceptable antidiabetic activity and oral bioavailability. Thus it will probably be a struggle for any small-molecule incretin mimetic to outperform emerging incretin peptide biologics in any of these categories other than offering the potential of oral administration.

Dual-acting peptides

Bayer pharmaceuticals have engineered a GLP-1/glucagon hybrid peptide that acts both as a GLP-1 agonist and as glucagon antagonist, named 'dual-acting peptide for diabetes' (DAPD) (Table 3).[74] This approach potentially benefits from blockade of the hyperglycaemic actions of glucagon, but may possibly increase the risk of unwanted hypoglycaemic episodes. Further preclinical development of DAPD has led to the formation of a PEGylated form (PEG-DAPD) with an improved plasma half-life in vivo.[74,75] PEG-DADP improves glucose tolerance and reduces blood glucose following a glucagon challenge.[75] Furthermore, PEGylation appears to eliminate the inhibitory effect of DADP on gastrointestinal motility, and it is hoped that this will prevent the more significant gastrointestinal side-effects such as nausea and vomiting. However, clinical studies are required to assess the efficacy of PEG-DADP and to determine how its performance compares with less promiscuous GLP-1 peptide mimetics.

CLINICAL DEVELOPMENT OF GLP-1 ANALOGUES/MIMETICS

Exendin/exenatide/Byetta

The first incretin-based pharmaceutical to reach the market was Byetta (Amylin/Eli Lilly), which is otherwise known as exendin-4(1–39), exenatide, or AC-2993 (Table 3). Byetta is a DPP-4-resistant GLP-1 receptor agonist isolated from the saliva of the Gila monster (*Heloderma suspectum*). Exenatide shares approximately 50% structural similarity with GLP-1 (see Figure 2). Extensive preclinical studies and more recent clinical studies show that exendin-4 replicates all of the known biological actions of GLP-1.[7,60] Since receiving approval by the US Food and Drug Administration in 2005, exenatide

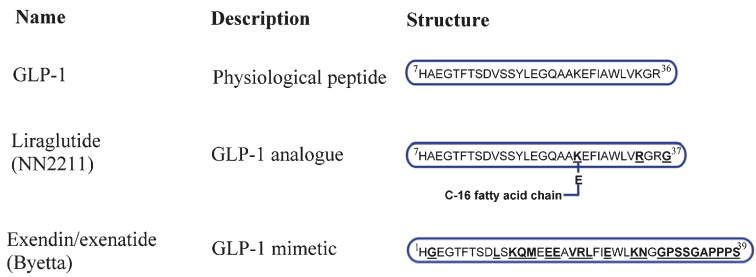

Figure 2. Structural representations of clinically relevant glucagon-like peptide 1 (GLP-1) analogues/mimetics: liraglutide (NN2211), a GLP-1 analogue, and exenatide (Byetta, exendin), a GLP-1 mimetic. Liraglutide has close structural similarity to GLP-1 but contains a C_{16} fatty acid chain attached at Lys^{26}. Originally isolated from the salivary glands of the Gila monster, exendin shares ~50% similarity with GLP-1. Exendin is resistant to degradation by dipeptidyl peptidase 4 (DPP-4) and is a potent agonist of the GLP-1 receptor. Underlined letters indicate amino acids which differ from native GLP-1.

has been launched in the USA and Europe. Exenatide requires twice-daily administration; a longer-acting formulation administered once weekly, known as exenatide LAR (long-acting release), is currently in phase-III trials (Table 3).

A summary of the medium- and long-term clinical studies for exenatide and exenatide LAR in type-2 diabetic patients is found in Table 4. Administration of exenatide 5–10 μg twice daily consistently lowered HbA1c values.[29–31,76,77] Furthermore, exenatide treatment often led to average weight losses of 1.5–2.8 kg.[29–31] Once-weekly administration (0.8 or 2 mg) of exenatide LAR to type-2 diabetic patients for 15 weeks lowered HbA1c (1.4–1.7%) and reduced body weight (up to 3.8 kg).[32] Although exenatide LAR was generally well tolerated, the most commonly observed side-effect was mild nausea.[32]

Liraglutide/NN2211

Liraglutide – Arg^{34},Lys^{26}-(N-ε-(γ-Glu(N-α-hexadecanoyl)))-GLP-1(7-37) – differs from exenatide in that it is a true analogue of GLP-1 and not simply a mimetic (Table 3). As shown in Figure 2, liraglutide is structurally similar to physiological GLP-1 but contains a C_{16} fatty acid chain attached to Lys^{26} which presumably orientates to mask the DPP-4 cleavage site. This modification alone appears to be sufficient to confer enzyme resistance, as is also observed with similar modifications to GIP.[63]

A summary of the medium-term clinical studies for liraglutide in type-2 diabetic patients is found in Table 5. Once-daily administrations of liraglutide across a range of doses (0.045–1.9 mg) led to dose-dependent reductions in fasting glucose and HbA1c.[28,78–80] In two studies, treatment with liraglutide for 12 weeks led to significant reductions in body weight.[28,81]

Other GLP-1 analogues

As can be seen in Table 3, there are several GLP-1 analogues/mimetics currently in preclinical/clinical development. Clinical data for other GLP-1 analogues are relatively scarce at present; however, one GLP-1 analogue developed by Eli Lilly, LY307161, has shown effective glucose-lowering properties when administered once daily for 21 days to type-2-diabetic subjects.[82] Conjuchem is developing two long-acting incretin compounds with chemical linkers. CJC-1331 and CJC-1134-PC are chemically modified forms of GLP-1 and exendin-4, respectively, with an ability to covalently bond with albumin following administration.[69,70] Both compounds have shown early promise in clinical trials (www.conjuchem.com). It is expected that these compounds will have protracted half-lives similar to those of liaraglutide and exenatide LAR, possibly being suitable for once-weekly administration. Little additional information is available concerning Albugon (aliglutide), the recombinant albumin–GLP-1 protein.[72]

CLINICAL POTENTIAL OF GIP

GIP agonists

Since in type-2-diabetic patients the action of GLP-1 on β cells is better preserved than that of GIP, most pharmaceutical effort in developing stable incretin mimetics has focused on GLP-1-receptor agonism. This endeavour has been fruitful (Table 3), but there is good reason to reconsider the clinical potential of GIP.[83] First, diminished

Table 4. Medium- and long-term clinical studies with exenatide (exendin, Byetta) and exenatide LAR (long-acting release) in type-2 diabetes.

Study	Compound	Dose	Duration (weeks)	Other intervention	n	Study design	Effect on glycaemic control
Fineman et al (2003)[76]	Exenatide	0.08 μg b.i.d 0.08 μg t.i.d	4	Sulphonylurea and/or metformin +	109	PC	↓ HbA1c 0.7–1.1%
Buse et al (2004)[77]	Exenatide	5–10 μg b.i.d.	30	Sulphonylurea	377	TBPC	↓ HbA1c 0.46% (5 μg) ↓ HbA1c 0.86% (10 μg)
Kendall et al (2005)[31]	Exenatide	5–10 μg b.i.d.	30	Metformin+ sulphonylurea	733	RDBPC	↓ HbA1c 1.0% ↓ BW 1.6 kg
DeFronzo et al (2005)[29]	Exenatide	5–10 μg b.i.d.	30	Metformin	336	RDBPC	↓ HbA1c 0.78% (5 μg) ↓ HbA1c 0.78% (10 μg) ↓ BW 2.8 kg (10 μg) ↓ BW 1.6 kg (5 μg)
Zinman et al (2007)[30]	Exenatide	10 μg b.i.d.	16	Thiazolidinedione	233	RDBPC	↓ FSG 1.7 mmol/L ↓ HbA1c 0.98% ↓ BW 1.5 kg
Kim et al (2007)[32]	Exenatide-LAR	0.8 or 2 mg once weekly	15	Diet or metformin	45	RPC	↓ HbA1c 1.4% (0.8 mg) ↓ HbA1c 1.7% (2 mg) ↓ FPG 2.4 mmol/L (0.8 mg) ↓ FPG 2.2 mmol/L (2 mg) ↓ BW 3.8 kg (2-mg dose only)

↓ decrease; PC, placebo-controlled; RDBPC, randomized double-blind placebo-controlled; RPC, randomized placebo-controlled; TBPC, triple-blind placebo-controlled; FSG, fasting serum glucose; FPG, fasting plasma glucose; BW, body weight.

Table 5. Medium- and long-term clinical studies with liraglutide (NN2211) in type-2 diabetes.

Study	Compound	Dose	Duration (weeks)	Other intervention	n	Study design	Effect on glycaemic control
Madsbad et al (2004)[28]	Liraglutide	0.045–0.75 mg/day	12	Glimepiride	193	RDBPC	↓ FPG 1.8 mmol/L ↓ HbA1c 0.75% ↓ BW 1.2 kg
Degn et al (2004)[78]	Liraglutide	6 μg/kg/day	1	Diet only	13	DBPCC	↑ HOMA-B 30% ↓ PPG 20%
Harder et al (2004)[79]	Liraglutide	0.6 mg/day	8	Diet only	33	RDBPC	↓ FSG 1.9 mmol/L ↓ HbA1c 0.33%
Feinglos et al (2005)[81]	Liraglutide	0.045–0.75 mg/day	12	Metformin	210	RDBC	↓ BW 1.9%
Nuack et al (2006)[80]	Liraglutide	0.5–2 mg/day	5	Metformin + Glimepiride	144	RDBC	↓ FSG 3.9 mmol/L ↓ HbA1c 0.8 % ↓ BW 1.5 kg
Vilsboll et al (2007)[106]	Liraglutide	0.625–1.9 mg/day	14	Diet only		RDBPC	↓ FPG ^{TBC} mmol/L ↓ PPG^{TBC} mmol/L ↓ HbA1c 1.7%

↓ decrease; DBPCC, double-blind placebo-controlled crossover; HOMA-B, homeostasis model assessment: β-cell function; RDBPC, randomized double-blind placebo-controlled; RDBC, randomized double-blind comparator; FSB, fasting serum glucose; FPG, fasting plasma glucose; PPG, postprandial plasma glucose; BW, body weight.

insulinotropic action of GIP is observed in studies using continuous infusion[11,12] but not simple bolus injections.[84] Second, it is recognized that the defect in GIP is acquired rather than representing a primary feature of type-2 diabetes.[85] Third, the defect is reversible, and GIP responsiveness can be restored clinically by sulphonylureas or by simply improving blood glucose control.[86–88] The involvement of hyperglycaemia in impairment of the GIP receptor has also been demonstrated in animals studies.[89] Finally, the proven efficacy of DPP-4 inhibitors, which act through potentiation of both GLP-1 and GIP action[54], indicates that these agents might partly or fully restore GIP responsiveness in humans. Taken together, accumulating evidence supports the therapeutic utility of GIP arising endogenously from DPP-4 inhibition or exogenously from injection of stable GIP analogues either alone or in combination with a GLP-1 mimetic. This latter approach is particularly interesting as it has established that the two incretin hormones potentiate each other's actions.[7]

As noted above, substantial basic and preclinical studies have been undertaken mainly in academic laboratories to identify a number of structurally modified forms of GIP as potentially attractive drug candidates. These include DPP-4-resistant N-terminally-modified and acylated fatty acid derivatised analogues of GIP (Table 2).[55] Amylin Pharmaceuticals has also recently reported development of a long-acting GIP analogue, AC163794, with antidiabetic activity.[90] Clinical studies are needed to assess the insulin-releasing and antihyperglycaemic potential of the most promising of these stable GIP receptor agonists in patients with type-2 diabetes. Since GIP does not inhibit gastric emptying in man[7], side-effects of nausea and vomiting exhibited by some of the longer-acting GLP-1 analogues may be less problematic.

GIP receptor antagonism

One of the most significant advances in incretin biology over the past few years is the increasing realisation that GLP-1 and GIP have actions outside of the pancreas and gastrointestinal tract. Anabolic effects on bone and possible cardio- and neuroprotective effects might also benefit patients with diabetes.[7] However, most significant is the presence of functional GIP receptors on adipocytes[91] and an appreciation that GIP secreted strongly in response to fat ingestion plays a major role in the translation of excessive amounts of dietary fat into adipocyte tissue stores[83,92], thereby impairing insulin action. Established effects of GIP on adipocytes include an increase in lipoprotein lipase, stimulation of lipogenesis, enhancement of fatty acid and glucose uptake, augmentation of insulin-induced fatty acid incorporation, and inhibition of both glucagon- and adrenergic-receptor-mediated lipolysis.

The above actions have opened up an unexpected therapeutic channel for exploiting GIP-receptor antagonism for treatment of obesity and associated insulin resistance.[54,83,92] Stated simply, at the level of the adipocyte, GIP promotes energy storage and reduces insulin action, whereas at the β cell GIP stimulates insulin secretion. Thus in well-established insulin-resistant obesity-related diabetes, the beneficial effects of GIP-receptor blockade take primacy and considerably outweigh the loss of the insulin-releasing GIP component of the enteroinsular axis.[93] This scenario is borne out by studies in high-fat-fed mice or *ob*/*ob* mice with either genetic knockout of GIP receptor[94,95] or induced chemical ablation of GIP action using the DPP-4-resistant and specific GIP receptor antagonist (Pro^3)GIP.[93,96,97] Most recent studies involving once-daily administration of (Pro^3)GIP to young *ob*/*ob* mice indicate that GIP-receptor blockade is also able to prevent the onset of diabetes.[97] A major observation

in these animal models is that by causing preferential oxidation of fat[98], GIP-receptor blockade is able to clear triglyceride deposits from liver and muscle, thereby respectively restoring mechanisms for suppression of hepatic glucose output and cellular glucose uptake.[96,97]

(Pro^3)GIP is the most potent N-terminally-modified GIP antagonist developed so far, effective by once-daily injection.[57,58,99] Knowledge of the C-terminal modifications used successfully to prolong the action of GLP-1 and GIP, such as acylation or PEGylation, will greatly assist the generation of second-generation longer-acting molecules. Further studies are clearly warranted to evaluate the potential of GIP-receptor antagonists and particularly their applicability to human obesity diabetes. However, as highlighted elsewhere[100], proof of concept is provided by emerging evidence indicating that rapid cure of diabetes in grossly obese subjects undergoing Roux-en-Y bypass surgery may be mediated by surgical bypass of GIP-secreting K cells in the upper small intestine. These individuals demonstrate low levels of circulating GIP (with compensatory increase in GLP-1) and the restoration of normoglycaemia due to substantial improvement of insulin resistance with accompanying β-cell glucose responsiveness.[101,102] Importantly, such effects precede any significant weight loss that will confer an additional metabolic advantage, as observed in genetic and diet-induced models of obesity diabetes.[96,97]

CLINICAL CONSIDERATIONS

Safety and tolerability

Currently available clinical data indicate that GLP-1 mimetics/analogues are generally well tolerated. Mild nausea is the most commonly reported side-effect, which is likely to be a result of the potent inhibitory effect of GLP-1 on gastric emptying.[27] Higher doses of liraglutide are reported to induce side-effects of nausea, vomiting, dizziness and headaches. Optimal dosing is necessary to minimise these side-effects.[103] Formulations with extended duration of action – e.g. exenatide LAR, administered once weekly – do not avoid side-effects. Nausea is less likely to occur with GIP analogues since this incretin hormone does not inhibit gastric emptying.[7]

Low titres of weak-affinity exendin antibodies were produced in 50% of patients following treatment[103], which is not surprising given that the Gila-monster peptide has only 50% sequence similarity to GLP-1. Usually this does not compromise the effectiveness of exenatide, although a few individuals with high-titre antibodies might be less responsive. Generally much higher doses of exenatide LAR are required compared with exenatide, typically 2 mg compared with 10 μg. It is not clear what the long-term consequences of this will be, but a 15-week trial reported increased frequencies of nausea, gastroenteritis, hypoglycaemia, and some injection-site bruising in subjects receiving exendatide LAR.[32] In contrast to exenatide, antibody production has not been detected following prolonged administration of liraglutide, a molecule with much closer structural similarity to GLP-1.

Overall, no serious adverse effects have been noted in the substantial numbers of type-2-diabetic patients following long-term administration of exenatide or liraglutide.[103] A high rate of nausea was encountered in early studies with CJC-1131, although CJC-1134-PC has been reported to lack major side-effects.[69,70] However, the lack of published clinical data on these and other agents, including DAPD and GIP analogues, makes their safety and tolerability difficult to assess at present.

Role in diabetes management

Exenatide is the first clinically approved agent in a class of new multi-action drugs which act through physiological mechanisms. It already shows great promise for treatment of type-2 diabetes cases which are not well controlled by oral agents. Accumulating experience with exenatide and other emerging agents will reveal whether stable incretins can also be considered for use as monotherapy and also possibly to delay or prevent the onset of type-2 diabetes. Positive effects of GLP-1 and GIP on the growth, survival and function of β cells also suggests a possible, but unproven, role in patients receiving islet transplantation. The extent of penetration of DPP-4 inhibitors into this same area will depend on their performance (efficacy and freedom from side-effects) as well as pharmaceutical marketing.

CONCLUSION

The diversity of useful actions of incretin hormones offers significant advantages over many existing antidiabetic drugs. These are being exploited through the development of incretin hormone analogues/mimetics. Strategies to extend the in vivo half-life of GLP-1 and GIP include: (1) modification/substitution of N-terminal amino acids; (2) attachment of molecules which facilitate plasma protein binding (e.g. acylation or PE-Gylation); and (3) direct fusion with plasma protein molecules. Such modifications confer resistance to degradation by DPP-4 and/or circumvent renal filtration. The first antidiabetic drug of this new class to be launched in the US and Europe was exenatide (Byetta/exendin), and phase-III clinical trials of an acylated GLP-1 analogue, liraglutide, appear promising. In extensive studies conducted to date, GLP-1 analogues/mimetics have produced sustained improvements in glycaemic control, with body weight loss, good tolerability, and few adverse effects. Currently exenatide is administered as a twice-daily subcutaneous injection; however, the prospect of a once-daily (liraglutide) or perhaps once-weekly (exenatide LAR) administration appears to be both feasible and attainable. A number of other GLP-1 analogues/mimetics are in clinical development, and advances in the quest for small-molecule GLP-1 agonists bring the possibility of oral therapy. Finally, recent research suggests that GIP-receptor antagonists may afford an entirely new drug class for alleviation of insulin resistance through entirely novel physiological pathways.

Practice points

- incretin hormone analogues/mimetics have proven efficacy and are a useful adjunctive therapy to oral agents
- there is good evidence that most patients taking exenatide or liraglutide will lose weight for as long as they are taking it
- incretin hormone analogues/mimetics act only in the presence of hyperglycaemia; episodes of hypoglycaemia can be reduced by lowering the dose of other oral antihyperglycaemic drugs
- incretin hormone analogues/mimetics are not recommended for use with insulin, since combination therapy carries an increased risk of hypoglycaemia

Research agenda

- new incretin formulations such as exenatide LAR allow the possibility of once-weekly instead of twice-daily administrations
- development of alternative routes of administration, such as oral or buccal tablets
- development of non-peptide incretin mimetics could potentially provide orally available administration
- further investigation of GIP-receptor antagonists might lead to the development of entirely new classes of drugs

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