Incretins: Their physiology and application in the treatment of diabetes mellitus

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Summary

Therapies targeting the action of incretin hormones have been under close scrutiny in recent years. The incretin effect has been defined as postprandial enhancement of insulin secretion by gut-derived factors. Likewise, incretin mimetics and incretin effect amplifiers are the two different incretin-based treatment strategies developed for the treatment of diabetes. Although, incretin mimetics produce effects very similar to those of natural incretin hormones, incretin effect amplifiers act by inhibiting dipeptidyl peptidase-4 (DPP-4) enzyme to increase plasma concentration of incretins and their biologic effects. Because glucagon-like peptide-1 (GLP-1) is an incretin hormone with various anti-diabetic actions including stimulation of glucose-induced insulin secretion, inhibition of glucagon secretion, hepatic glucose production and gastric emptying, it has been evaluated as a novel therapeutic agent for the treatment of type 2 diabetes mellitus (T2DM). GLP-1 also manifests trophic effects on pancreas such as pancreatic beta cell growth and differentiation. Because DPP-4 is the enzyme responsible for the inactivation of GLP-1, DPP-4 inhibition represents another potential strategy to increase plasma concentration of GLP-1 to enhance the incretin effect. Thus, anti-diabetic properties of these two classes of drugs have stimulated substantial clinical interest in the potential of incretin-based therapeutic agents as a means to control glucose homeostasis in T2DM patients. Despite this fact, clinical use of GLP-1 mimetics and DPP-4 inhibitors have raised substantial concerns owing to possible side effects of the treatments involving increased risk for pancreatitis, and C-cell adenoma/carcinoma. Thus, controversial issues in incretin-based therapies under development are reviewed and discussed in this manuscript. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords incretins; GLP-1; GLP-1 analogues; DPP-4 inhibitors; diabetes

Introduction

Currently, 371 million people have been reported to have diabetes in the world, and 90% of them has type 2 diabetes mellitus (T2DM) [1]. Increase in obesity rates has been correlated with an increase in the prevalence of diabetes. Diabetes is now considered to be the world’s biggest pandemic disease with a prevalence of 8% [2]. Five million people have died in 2011 because of the secondary complications of diabetes including coronary heart disease and peripheral vascular diseases [3]. Furthermore, diabetes has been
reported to be the most important cause of blindness and renal failure in developed countries.

Although diabetes is primarily managed by lifestyle changes and dietary modifications, administration of a pharmacological agent is required especially when treatment goals are not achieved. These conventional treatment agents include but not limited to biguanides, sulfonylureas, thiazolidinediones, meglitinides, alpha-glucosidase inhibitors and insulin along with a recently developed amylin analogue pramlintide [4]. Current guidelines recommend biguanide metformin as a first-line treatment, with subsequent addition of other agents when this monotherapy is no longer effective [5]. Despite intensive therapy, glycaemic control can still be lost, leading to an increase in HbA1c levels of diabetic patients. Moreover, current therapies are often associated with weight gain and hypoglycaemia [6]. Other adverse events include but not limited to gastrointestinal discomfort with the use of biguanides, and possible oedema, cardiac failure or fractures due to the use of thiazolidinediones.

Obesity, insulin resistance and beta cell malfunction eventually leading to beta cell loss are the prominent features of T2DM; development of novel anti-hyperglycaemic agents with the least side effects requires a detailed understanding of the pathophysiology of diabetes. In this context, blood glucose is mainly controlled with the combined actions of insulin and glucagon in association with liver, muscle and adipose tissues. After the ingestion of a meal, gut-derived factors are secreted to enhance insulin discharge from pancreatic beta cells [6,7]. These gut-derived factors that enhance glucose-stimulated insulin secretion from islet beta cells are called incretins. In this scenario, oral glucose administration promotes a greater degree of insulin secretion compared with parenteral-isoglycaemic glucose infusions [8]. Although carbohydrates, protein and fat all contribute to the secretion of incretins to some degree, carbohydrates is the most effective agent in causing incretin secretion. This is because carbohydrate absorption is the only way to increase glucose levels in circulation, and incretins stimulate insulin secretion only when blood glucose is high.

Although incretins are crucial in the maintenance of normoglycaemia by way of facilitating glucose transport into peripheral tissues [9], T2DM patients displayed insulin resistance and reduced incretin secretion, resulting in ineffective glucose clearance from circulation. Because reduced incretin response to food ingestion is one of the primary defects associated with glucose intolerance and hyperglycaemia in T2DM, incretin-based treatment strategies recently gained a significant momentum as a novel class of medications with anti-diabetic potential as discussed in this manuscript.

Molecular structure and secretion of incretins

The first incretin hormone was initially named as gastric inhibitory polypeptide (GIP) because it inhibited gastric acid secretion in dogs [10]. Later, this peptide was renamed as glucose-dependent insulinotropic polypeptide owing to its insulinotropic effect observed at physiological doses. The cloning and the sequencing of the mammalian proglucagon gene resulted in the discovery of a second incretin hormone, glucagon-like peptide-1 (GLP-1) [11]. GLP-1 is produced from the flask-shaped L cells in the distal jejunum, ileum and colon, while GIP is secreted from K cells localized to the proximal intestinal mucosa (duodenum and upper jejunum). Apart from these proximally (K cells) and distally located (L cells) cell types, the existence of mixed cell populations synthesizing GIP and GLP-1 throughout the small intestine has been reported as well [12]. Consequently, post-translational processing of the proglucagon polypeptide by the prohormone convertase 1/3 [13,14] results in the production of GLP-1, GLP-2, oxyntomodulin and glicentin (Figure 1). Intriguingly, only GLP-1 is capable of augmenting insulin secretion in response to glucose.

Glucagon-like peptide-1 is first synthesized as an inactive 37 aa polypeptide (GLP-11–37) with a glycine at the carboxyl terminus. Post-translational processing of

![Figure 1. Processing of proglucagon and proGIP by PC1/3 generates incretins with insulinotropic effect in intestine. For this to happen, proglucagon is first processed to produce glicentin, GLP-11–37 or GLP-11–36 amide, IP2 and GLP-2. Glicentin and GLP-1 can further be cleaved by PC1/3 to yield oxyntomodulin and GLP-15–37 or GLP-15–36 amide, respectively. Similarly, proGIP is modified by PC1/3 to generate GIP1–42 in intestine. Abbreviations: GRPP, glicentin-related pancreatic polypeptide; GLP-2, glucagon-like peptide-2; PC, prohormone convertase; IP, intervening peptide; GIP, gastric inhibitory polypeptide](image-url)
GLP-1 results in the removal of six aa from its amino terminus, yielding biologically active peptide. Bioactive GLP-1 in circulation exists as GLP-1\(_{7-37}\) and GLP-1\(_{17-36}\) amide, and GLP-1\(_{17-36}\) amide is the most abundant form of active GLP-1 in human plasma [15]. GLP-1 is inactivated by dipeptidyl peptidase-4 (DPP-4) producing GLP-1\(_{9-36}\) amide and/or GLP-1\(_{9-37}\) [16]. Because DPP-4-mediated GLP-1 processing is so fast, most of the detectable immuno-reactive GLP-1 in circulation and in the portal vein are in truncated forms. Despite GLP-1\(_{7-37}\) and GLP-1\(_{17-36}\) amide being the biologically active forms of GLP-1, recent studies showed the existence of other processed shorter forms with some additional biologic activities [17]. Likewise, amino-truncated forms of GLP-1, GLP-1\(_{19-36}\) amide and GLP-1\(_{19-37}\), have been claimed to play crucial roles in cardio-protection and cell viability [18-20].

Gastric inhibitory polypeptide is a 42-aa peptide synthesized from proGIP by way of prohormone convertase 1/3 and secreted by the duodenal K cells located in the upper small intestine (Figure 1) [21]. The presence of an alanine in the second position leads to its quick degradation by DPP-4. Thus, right after its secretion from intestinal K cells, GIP\(_{1-42}\) is converted into GIP\(_{3-42}\). Although a physical contact between the nutrients and proximal K cells is required for GIP secretion [22], no such requirement for GLP-1 secretion exists because nutrients are digested long before they reach the L cells within the distal intestine. In other words, right after the food intake but before the passage of digested nutrients into the small intestine, distal L cells release GLP-1 under the influence of neuronal and endocrine factors such as vasoactive intestinal peptide and pituitary adenylate cyclase-activating peptide [23,24]. Currently, GLP-1-induced and GIP-induced incretin response is responsible for 70% of postprandial glucose-dependent insulin secretion [16].

**Signalling mechanisms of incretin action**

Human GLP-1 receptor (GLP-1R) is a G-protein-coupled receptor (GPCR) synthesized in pancreatic islets along with the kidney, lung, heart and nervous system (Figure 2). Similarly, GIP receptor (GIPR) is synthesized in pancreatic islets besides adipose tissues, heart and brain, stimulating similar signalling pathways induced by GLP-1. Despite incretins enhancing glucose-dependent insulin secretion, the mechanism that cause GLP-1 and GIP to induce insulin secretion only under high plasma glucose is not known. Although both incretins cause cyclic adenosine monophosphate (cAMP) production and activate protein kinase A (PKA), PKA inhibitors cannot completely block the insulinotropic activities of these two peptides [25]. PKA-independent insulinirotropic effects have been attributed to the activities of guanine nucleotide exchange factors, in particular to exchange protein directly activated by cAMP [26]. GLP-1 reduces blood glucose through inhibition of glucagon secretion from pancreatic alpha cells. Because GLP-1R-mediated suppression of glucagon secretion is dependent on plasma glucose, as glucose level returns to normal, GLP-1 inhibitory signal from alpha cells is removed, preventing further development of hypoglycaemia [27,28].

Contrary to insulinotropic agents acting through \(K_{ATP}\) channels, GLP-1 is also involved in the refreshment of the intracytoplasmic insulin depots through enhancement of cAMP-mediated proinsulin gene transcription and mRNA stabilization [11]. To accomplish this task, GLP-1 stimulates Pdx-1 gene synthesis and its binding to insulin gene promoter [29]. Thus, a decrease/loss of Pdx-1 gene expression results in either attenuation or impairment of GLP-1 function in pancreatic beta cells [30,31]. Consequently, reduced Pdx-1 gene synthesis was correlated with GLP-1R deficiency or insufficient GLP-1R agonist (exendin-4) response [30]. Furthermore, Pdx-1 gene synthesis is also responsible for the anti-apoptotic properties of GLP-1R agonists on beta cells. As a result, beta cell-specific Pdx-1 gene knockout mice exhibited increased beta cell apoptosis and were unresponsive to exendin-4 treatment [31].

Because GLP-1 or exendin-4 treatment in neonatal Wistar rats promoted beta cell regeneration, increasing beta cell mass [32], GLP-1R agonists could induce beta cell proliferation even in normoglycaemic animals [33-35]. Moreover, GLP-1R agonist-induced upregulation of Pdx-1 gene synthesis resulted in an increase in the beta cell mass of diabetic mice [36]. Hence, neonatal administration of exendin-4 was able to restore beta cell loss in intrauterine growth-retarded rats [37]. The mechanism for GLP-1-mediated and exendin-4-mediated increase in beta cell mass was attributed to the inhibition of apoptotic signalling cascades as demonstrated in db/db [38] and streptozotocin-injected mice [39]. In addition, GLP-1 induced differentiation of human islet progenitor cells into functional beta cells by enhancing Pdx-1, glucokinase and glucose transporter 2 gene synthesis [40]. In the absence of Pdx-1 gene synthesis, however, PANC-1 cells failed to differentiate [41]. Just like GLP-1, GIP manifested both proliferative and anti-apoptotic effects on pancreatic beta cells [42,43].

**Physiologic effects of incretins**

The physiologic effects of incretins were revealed by studies performed either in mice lacking incretins or using...
incretin peptide antagonists in various species including humans (Table 1). GLP-1R antagonist, exendin-9–39 amide, increased both fasting and postprandial blood glucose owing to reduced insulin secretion in treated subjects [44–47]. High plasma glucagon levels [48] and accelerated gastric emptying [49, 50] were observed in exendin-9–39 amide-treated patients, demonstrating that GLP-1 is a tonic inhibitor of glucagon secretion with the potential to decelerate gastric emptying. Moreover, inactivation of GLP-1R reduced both oral and intraperitoneal glucose-induced insulin secretion, resulting in impaired glucose tolerance [51, 52]. Although GLP-1 exhibited a positive effect on satiety and weight loss, feeding high-fat diet did not alter eating behaviour or cause weight gain in GLP-1R−/− mice [53]. In addition, GLP-1R−/− mice displayed learning difficulties, epileptic sensitivity and impaired myocardial contraction [54, 55].

Likewise, the functional role for GIP on glucose homeostasis was studied using GIP antagonists and GIPR blocking antisera (Table 1) [56]. Although GIP played a significant role in reducing postprandial glucose excursion, GIP-1R antibody treatment failed to alter plasma glucose or circulating insulin in mice [47]. In addition, targeted disruption of GIP-1R caused only a minor glucose intolerance in response to oral glucose challenge [57]. As GIPR−/− mice manifested no alteration in body weight, mice fed with a high-fat diet displayed reduced adipocyte fat mass accompanied by a resistance to diet-induced obesity [58]. Although chronic use of a GIPR antagonist (Pro3-GIP) impaired glucose tolerance in wild-type mice [59], daily Pro3-GIP treatment decreased both plasma glucose and insulin in addition to enhancing insulin sensitivity in ob/ob mice [60]. Moreover, chemical ablation of GIPR slowed down the development of islet cell hypertrophy and beta cell hyperplasia in ob/ob mice.

Figure 2. Molecular signalling mechanism responsible for the insulinotropic effects of glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP). Interaction of GLP-1 and GIP with their cognate receptors, GLP-1R and GIPR, results in the activation of adenylate cyclase (AC) by way of G proteins (G) leading to increase in intracellular cyclic adenosine monophosphate (cAMP) levels. Activation of protein kinase A (PKA) and exchange protein directly activated by cAMP (cAMP-GEFII) closes KATP channels (K Ch) facilitating membrane depolarization resulting in the opening of the voltage-gated Ca2+ channels (Ca Ch) and influx of Ca2+ into pancreatic beta cells. Increase in cytoplasmic Ca2+ not only stimulates fusion of insulin-containing cytoplasmic granules leading to insulin secretion from pancreatic beta cells but also promotes transcription of proinsulin gene refreshing insulin depots. Key players of glucose-mediated insulin secretion are also depicted in the figure. In this scenario, glucose enters the cell through glucose transporter-2 (GLUT2) and gets phosphorylated to glucose 6 phosphate (G6P) by glucokinase (GK). Glycolysis increases the adenosine triphosphate (ATP)/adenosine diphosphate ratio, leading to closure of K channels (K Ch) inducing membrane depolarization. Other abbreviations: Nu, nucleus; ER, endoplasmic reticulum, Mt, mitochondria.
One of the most prominent effects of GLP-1 in postprandial glucose management is to slow down gastric emptying as observed minutes after the administration of a pharmacologic GLP-1R agonist. This is accomplished by a complex communication bridge between the central and peripheral nervous systems. Interestingly, gastric distension increased GLP-1 synthesis in the brain stem [61]. GLP-1 has also been considered to be a brain–gut peptide modulating gastric motility, as removal of vagal afferent neurons inhibited GLP-1-mediated delay in gastric emptying [49]. Although GLP-1 and exendin-4 could pass through the blood–brain barrier, reaching the central nervous system, large GLP-1R agonists that were unable to accomplish this passage could still slow down gastric emptying, reducing food intake [62]. Thus, vagal afferent neurons extending to the central nervous system play an important role in GLP-1-dependent gastrointestinal motility. Because GLP-1R is localized to hypothalamic nucleus controlling satiety, both intracerebroventricular injection (ICV) and peripheral administration of GLP-1 agonists were able to reduce food intake [63,64]. Although repeated ICV injections of GLP-1 produced weight loss in rats, delivery of GLP-1R antagonist exendinα-36 antagonized this effect, causing weight gain [65]. Administration of chronic peripheral GLP-1R agonist also resulted in reduced food intake, generating weight loss in other preclinical studies [66,67].

Glucagon-like peptide-1 has been reported to improve endothelial function in patients with T2DM [68]. Similarly, GLP-1 enhanced myocardial function and cardiac performance in patients with acute myocardial infarction and left ventricular dysfunction [69]. In addition, GLP-1 reduced the infarct area in a myocardial ischaemia model [18,70]. The cardioprotective effects of GLP-1 could be inhibited by cAMP inhibitor Rp-cAMP, phosphatidylinositol 3-kinase inhibitor LY294002 and p42/44 mitogen-activated protein kinase inhibitor UO126 [71]. However, it is not known whether direct GLP-1R signalling or alteration in GLP-1R-dependent glucose-induced insulin secretion is responsible for the cardioprotective effects of GLP-1.

Another way to increase the GLP-1 and GIP concentrations in blood is to block DPP-4 activity, which inactivates these two peptides by truncating them from the second aa (alanine) [72]. Selective and nonselective DPP-4 inhibitors were utilized, and DPP-4 gene mutant rodent models were generated to reveal the biological importance of DPP-4. Chemical inhibitors of DPP-4 prevented GLP-1 and GIP inactivation as demonstrated in many preclinical [73] and clinical studies [74]. Likewise, DPP-4-deficient Fischer 344/CRJ rats displayed increased plasma GLP-1 with improved glucose tolerance [75]. Similarly, targeted inactivation of DPP-4 produced insulin sensitivity and established resistance to diet-induced obesity by way of increasing plasma GLP-1 and insulin [76,77]. In addition, DPP-4 inhibition in T2DM patients prevented weight gain, increased beta cell function and suppressed glucagon secretion with a decrease in HbA1c [78]. However, DPP-4 inhibitors were not effective in reducing the blood glucose of double incretin receptor knockout mice [79]. Because high-dose injection of selective DPP-4 inhibitors did not interfere with the activation of T cells, these inhibitors are considered to be safe for clinical use [80].

**Pathophysiology in type 2 diabetes mellitus**

A 50% reduction in incretin response was detected in T2DM patients in comparison with healthy individuals.
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Therapeutic applications

Two treatment strategies that are essential to retain the therapeutic effects of GLP-1 are under development. These are GLP-1R activators also known as incretin mimetics and incretin effect amplifiers (DPP-4 inhibitors) (Figure 3) [92].

Glucagon-like peptide-1 mimetics

The substitution of the second aa of GLP-1 to any other aa such as glycine (alanine to glycine) makes GLP-1 resistant to DPP-4 without compromising its biologic activity [93]. Despite this modification, its clinical efficacy is still limited owing to its quick removal by kidneys, with a plasma half-life of 4–5 min. On the other hand, exendin-4 (exenatide) isolated from the venom of Gila monster (Heloderma suspectum) with a 50% sequence homology to GLP-1 is an ideal activator (agonist) of GLP-1R (Figure 3). Because the second aa of exendin-4 is glycine, it is already resistant to DPP-4 degradation [67]. Although it is eliminated from the body through glomerular filtration, exenatide exhibits a half-life of 30 min in circulation [94]. Because a single dose of subcutaneous injection of exenatide provides 5–6 h of insulinotropic action in plasma, an injection twice per day is sufficient to take advantage of its anti-diabetic properties [95]. One single subcutaneous injection of the long-acting release form of exenatide, consisting of exenatide contained within a poly-lactide-glycolide microsphere suspension, provided 28 days of glucose control as demonstrated in Zucker diabetic fatty rats [96]. As exendin-4 completely mimicked the glucose-lowering effects of GLP-1, an injection twice per day was sufficient to lower HbA1c levels by 1% [97]. The therapeutic efficacy of exendin-4 was tested in another study for 30 weeks in T2DM patients, who were unresponsive to metformin or sulfonylurea treatment. As exendin-4 successfully decreased HbA1c levels by 0.9% with a benefit of weight loss, nausea was the only observable side effect of the treatment [98,99]. In addition, the clinical efficacy of exendin-4 in combination with insulin glargine has been investigated in T2DM patients unresponsive to oral anti-hyperglycaemic agents with starting HbA1c levels of 8.2%. Although exendin-4 and insulin glargine treatments resulted in preferential reduction of either postprandial glucose levels (with exendin-4) or fasting blood glucose (with insulin glargine), the combined treatment effectively reduced HbA1c levels by 1.1% [100]. Intriguingly, side effects such as nausea, vomiting and diarrheas were more common in patients treated with exendin-4 compared with patients treated with insulin glargine. Additionally, while insulin glargine-treated patients gained 1.8 kg on average, exenatide treatment yielded

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Table 1: GLP-1 mimetics and incretin effect amplifiers (DPP-4 inhibitors).
2.3 kg weight loss per patient. In this context, exenatide (Byetta) was the first incretin mimetics marketed by Amylin and Eli Lilly. It is formulated for subcutaneous injection to be delivered before the breakfast and evening meals. Currently, exenatide is recommended as adjunctive therapy to manage blood sugar in T2DM patients, who are unresponsive to metformin and/or sulfonylurea.

Liraglutide (Victoza) is another long-acting incretin mimic (GLP-1 agonist) developed by Novo Nordisk (Figure 3). The European Medicines Agency (EMEA) approved its use on 3 July 2009, and the US Food and Drug Administration (FDA) on 25 January 2010. Liraglutide has arginine instead of lysine at position 28, and a C-16 fatty acid chain (palmitic acid) is attached to K20 with a glutamic acid spacer in order to delay its removal by kidneys [101]. A single use per day is recommended owing to slow absorption after subcutaneous injection, with a half-life of 11–13 h [102]. Liraglutide exhibited clinical efficacy similar to that of exenatide, reducing HbA1c levels by 1% and lowering body weight by 2.3 kg without developing hypoglycaemia in T2DM patients [103,104]. Pancreatic beta cell functions were also increased in liraglutide-treated patients. Despite the fact that 57% of the patients displayed nausea and 17% vomiting, gastrointestinal side effects were mainly dose dependent.

**Incretin effect amplifiers (DPP-4 inhibitors)**

Dipeptidyl peptidase-4 also known as T-cell antigen, CD26, is a serine peptidase present in plasma, kidneys, intestinal mucosa, hepatocytes and vascular endothelial cells [105]. DPP-4 removes two aa from the amino terminus of the target peptides including GLP-1 and GIP, resulting in their inactivation [106]. DPP-4 also plays a key role in T-cell activation and proliferation. However, this particular effect of DPP-4 on the immune system is independent of its enzymatic activity [107]. Because GLP-1 is quickly truncated and inactivated by DPP-4 in circulation, the use of DPP-4 inhibitors was necessary to maintain certain GLP-1 concentration in blood to maintain a therapeutic effect [90]. DPP-4 inhibitor-mediated enhancement of the insulinotropic and anti-hyperglycaemic
actions of GLP-1 [93] and GIP [108] was initially demonstrated in anaesthetized pigs. Clinical use of DPP-4 inhibitors has been reported to be safe and tolerable without compromising the immune system in T2DM patients. The first clinical study concerning the use of first-generation DPP-4 inhibitors (Novartis DP728) for 4 weeks demonstrated that fasting blood glucose and HbA1c could effectively be reduced in T2DM patients [109]. Furthermore, the deterioration of glycaemic control could be prevented with 1% reduction in HbA1c levels in T2DM patients as shown in a 12- to 52-week clinical trial of DPP-4 inhibitor, LAF237, in conjunction with metformin therapy [74].

Sitagliptin was the very first marketed DPP-4 inhibitor (Januvia, Merck) [110]. Entrance of the second DPP-4 inhibitor, Vildagliptin (Galvus, Novartis), to European market took place in the spring of 2008 (Figure 3). These two DPP-4 inhibitors are taken as oral tablets and provide 70–90% inhibition in DPP-4 activity lasting 24 h when administered as a single dose. Intriguingly, vildagliptin, similar to other GLP-1 mimetics, induced peripheral insulin sensitivity, enhanced glucose-induced insulin secretion from beta cells and suppressed glucagon secretion [111,112]. Because a single 100-mg dose of vildagliptin caused an increase in hepatic transaminases, 50-mg vildagliptin twice-a-day formulation was recommended for use. However, unlike incretin mimetics, DPP-4 inhibitors do not generate weight loss. Because metformin enhanced GLP-1 biosynthesis and secretion, DPP-4 inhibitors were more effective when they were used in combination with metformin [113]. In addition, DPP-4 inhibitors could also be used in conjunction with insulin therapy [114]. In conclusion, despite the fact that sitagliptin and vildagliptin monotherapies exhibited significant anti-diabetic properties, they were more effective in reducing hyperglycaemia when they were used in combination with other anti-diabetic agents such as metformin, sulfonylurea and thiazolidinediones.

Novel incretin-based experimental approaches under development

One of the advantages of using DPP-4 inhibitors is the availability of oral tablets for drug delivery. Unfortunately, current GLP-1R agonists require injections because they are not available in tablet forms. Thus, orally taken GLP-1R agonists are currently under development. By this token, intratracheal delivery of an 11-mer GLP-1R peptide agonist (inhaleable, spray-dried powder formulation), BMS-686117, to the lungs of rats resulted in 45% bioavailability and rapid onset of action relative to subcutaneous injection [115]. As a non-peptidic oral GLP-1R agonist, Boc5, invoked sustained glycemic control and weight loss in diabetic db/db mice [116,117], DPP-4-resistant GLP-1-attached micro-beads (a modified polymer preparation) also improved glucose tolerance in diabetic db/db animals [118]. The fact that carrier-bound oral GLP-1 peptide enhanced glucose-induced insulin secretion in humans further supported these findings [119]. Additionally, buccal GLP-1 tablets were relatively effective in increasing plasma insulin in T2DM patients [120]. Despite these results, pharmacokinetic, safety and efficacy studies regarding oral GLP-1 tablets for human use have to be studied further.

Analogs with amino acid or N-terminal group modifications, DPP-4-resistant agonists and fusion proteins conjugating GLP-1 to other peptides have been developed to prolong the plasma half-life of GLP-1 [34]. Novel DPP-4-resistant GLP-1 peptides have been generated with substitution of the Ala8 residue of GLP-1 by either valine or 2-amino-butyric acid [121]. Despite these alterations, generated new compounds were still quickly removed from the blood by way of renal clearance. GLP-1 and exendin-4 mutants carrying disulfide bonds with increased half-lives induced better glucose tolerance and higher HbA1c reduction compared with their native unmodified forms in rodents [122]. Direct conjugation of GLP-1 analogues to albumin [123], attachment of an albumin-binding fatty acid to GLP-1 [124] and creation of recombinant albumin-GLP-1 fusion protein [62] were the other avenues explored to take advantage of the long half-life of serum albumin. Albiglutide was produced as a fusion of human albumin to two copies of DPP-4-resistant GLP-1 analogues with an extended half-life of 5 days [125]. Despite the fact that weekly and biweekly administration of albiglutide improved glucose tolerance in diabetic patients, nausea, vomiting, headache, dizziness, nasopharyngitis, back pain, influenza, upper respiratory tract infections and local skin reactions were reported as adverse events. Modified GLP-1 peptides excluding albiglutide still had a half-life of only a few hours when given subcutaneously, requiring daily administration. In some cases, addition of other oral anti-diabetic agents was needed to normalize blood glucose as demonstrated in many clinical trials. Because patient compliance is a key component of diabetes management and repeated daily injection is a significant hurdle, the design and the production of longer-acting molecules with the native GLP-1 actions were necessary.

Because GIP is an incretin hormone, blockage of GIPRs reduced postprandial insulin release [56]. However, GIP could not manifest insulinoergic effects in T2DM patients [126]. Furthermore, targeted destruction of GIP-secreting K cells [127] and GIPR-knockout mice [57] displayed resistance to diet-induced obesity. Thus, the therapeutic outcome of constant obstruction of GIPR has been studied in obesity-induced diabetes (Table 1). (Pro3) GIP-mediated blockage of GIPR alleviated obesity, insulin
resistance and diabetes associated metabolic complications (blood glucose, HbA1c, and insulin) in mice fed with high-fat diet [128]. An active vaccination approach using GIP peptides covalently attached to virus-like particles was also employed to interfere with GIP signalling [129]. Vaccination-induced GIP blockage induced resistance to diet-induced obesity with no signs of glucose intolerance. Consequently, long-term inhibition of GIPR signalling might be an effective treatment option for obesity-related diabetes.

One of the experimental approaches in T2DM is to enhance incretin secretion by way of GPCR in K and/or L cells. Three of these receptors, GPCRs 40, 119 and 120, have been isolated and purified from these cell types [130,131]. While activation of GPCR-40 enhanced fatty acid-dependent insulin secretion [132], GPCR-40-knockout mice displayed reduced incretin response to high-fat diet [133]. In addition, as the GPCR-119 agonist augmented glucose-induced insulin secretion in mice [134], oral GPCR-119 agonist administration enhanced both GLP-1 and GIP secretions [135]. Moreover, GPCR-120 served as a receptor for dietary unsaturated long-chain fatty acids in stimulation of GLP-1 secretion [136]. As a result, fatty acid receptor agonists could enhance insulin secretion directly through beta cells as well as indirectly by way of enhancing incretin secretion from intestinal cells [137].

**Treatment targets in incretin-based therapy**

**Reduced incretin effect**

Insulinotropic actions of exogenous incretin hormones GIP and GLP-1 \( ^{\text{36}} \) amide were compared in nine T2DM patients and in nine age-matched and weight-matched healthy subjects [126]. GLP-1 \( ^{\text{36}} \) amide but not GIP exhibited insulinotropic activity with a glucagonastic effect in the mild form of T2DM. Moreover, GLP-1 \( ^{\text{36}} \) amide reduced blood glucose, inhibited glucagon secretion and slowed down gastric emptying in T2DM patients [138]. Similar studies were conducted with GIP, involving 31 normal subjects and 68 newly diagnosed T2DM patients [139]. An exaggerated GIP response to oral glucose challenge and mixed meals was obtained in T2DM patients. As reported previously, incretin effect is reduced in T2DM [140]. The plasma concentrations of intact biologically active GLP-1 and GIP were measured after a mixed breakfast meal in 12 T2DM patients (body mass index of 31 kg/m\(^2\) and HbA1c of 9.2\%) and 12 healthy controls [141]. The late GLP-1 response, but not GIP secretion, was strongly reduced in T2DM patients, supporting the hypothesis that an impaired GLP-1 function contributed to the ineffective insulin secretion. To elucidate the mechanism of reduced incretin effect, the secretion of incretin hormones GLP-1 and GIP was investigated during a 4-h mixed-meal test in 54 T2DM patients, 33 matched control subjects with normal glucose tolerance and 15 unmatched subjects with impaired glucose tolerance [142]. As patients with impaired glucose tolerance were hyperinsulinaemic and generally showed similar metabolic abnormalities to diabetic patients, the meal-related GLP-1 secretion, but not GIP response, was severely reduced in T2DM patients. To investigate whether the reduced incretin effect observed in T2DM patients was a primary event in the pathogenesis of T2DM or a consequence of the diabetic state, eight patients with chronic pancreatitis and secondary diabetes, eight patients with chronic pancreatitis and normal glucose tolerance, eight patients with T2DM and eight healthy subjects were studied [81]. The incretin effect was significantly reduced in patients with chronic pancreatitis and secondary diabetes than in patients with chronic pancreatitis and normal glucose tolerance. Hence, the reduced incretin response was not a primary event in the development of T2DM but was rather due to the consequence of the diabetic state. Nevertheless, GLP-1 secretion, but not GIP response, was reduced in T2DM patients [143]. Consequently, GLP-1 injection, but not GIP administration, enhanced glucose-induced insulin secretion, suppressed glucagon secretion and delayed gastric emptying in T2DM patients.

**Weight gain**

Patients with T2DM are generally overweight, and most of the anti-hyperglycaemic agents except biguanides are not effective in causing weight loss. Instead, anti-hyperglycaemic agents such as sulfonylurea and insulin contribute to weight gain in diabetic patients, complicating the treatment efficacy or even worsening the prognosis of diabetic patients.

**Impaired beta cell function and beta cell loss**

Glucose tolerance is usually lost long before the actual appearance of T2DM. The UK Prospective Diabetes Study Group stated that a 50% decrease in beta cell function and 40% loss in islet cell mass have been observed in newly diagnosed T2DM patients [144]. Hyperglycaemia-induced glucotoxicity and lipotoxicity associated with an increase in unesterified fatty acids in circulation resulted in the functional loss of beta cell. The fact that no hyperglycaemia has been reported without the functional loss of beta cells further supported this hypothesis.
Therefore, beta cell function and mass must be restored to prevent progression of diabetes.

**Rapid gastric emptying**

Rapid gastric emptying led to the inability to control blood glucose in T2DM patients as demonstrated in nine newly diagnosed T2DM patients and nine sex-matched and age-matched non-diabetic control subjects [145]. Therefore, deceleration of gastric emptying is helpful to manage glucose excursions after feeding, reducing hyperglycaemia. By this token, amylin (pramlintide) and incretin mimetics (GLP-1) represent two options with beneficial effects in T2DM.

**Hyperglucagonaemia**

Patients with T2DM generally manifest high glucagon levels [146]. On top of that, high glucagon is the sign of impaired glucose tolerance. Consequently, high glucagon in T2DM patients stimulates glucose release from liver, enhancing hyperglycaemia. Although there are some experimental treatment approaches targeting hyperglucagonaemia, currently used treatment strategies do not involve inhibition of glucagon secretion.

**Effects of incretin treatment including side effects**

Although incretins have been first proposed for the treatment of T2DM in 1992, the first incretin hormone (GLP-1) for commercial use was approved in 2005. One of the main reasons for the delayed approval was the short half-life of GLP-1, which is less than 2 min, requiring constant infusion or frequent injection to maintain its insulinotropic activity. Intriguingly, less than 10% of the administrated GLP-1 is intact and biologically active only minutes after the injection [147]. To overcome this problem, two strategies have been proposed concerning the development of either DPP-4 resistant GLP-1 mimetics or DPP-4 inhibitors. Incretins enhance glucose-induced insulin secretion through interaction with GPCRs on pancreatic beta cells [148]. However, incretins cannot exhibit their insulinotropic effect at low glucose concentration under 4 mM, necessary to prevent development of hypoglycaemia. In addition, GLP-1 stimulates both gene expression and biosynthesis of insulin. Stimulation of beta cell proliferation and differentiation and inhibition of beta cell apoptosis are the other beneficial effects of GLP-1 [149]. Apart from causing weight loss through inhibition of appetite and food intake [88] and deceleration of gastric emptying [150], GLP-1 enhanced myocardial performance, reduced infarct area and restored endothelial functions in T2DM patients [151]. Lastly, GLP-1 mimetics increased plasma GLP-1 concentration better than what was achieved with DPP-4 inhibitors alone. The GLP-1 analogue exenatide can be injected twice daily before meals or once weekly when given within dissolvable poly-(D,L-lactide-co-glycolide) microspheres [152].

Despite all these beneficial effects of GLP-1, there are considerable numbers of concerns relating to the side effects of incretin-based therapy.

For example, exenatide and liraglutide have been reported to cause significant gastrointestinal discomfort in T2DM patients [153,154]. Because exendin-4 (exenatide)-based treatment strategies are antigenic, it is not clear if this would limit the clinical efficacy of GLP-1 mimetics [155]. The other side effects of GLP-1 mimetics include but not limited to nausea, vomiting and hypoglycaemia [156]. Severe side effects leading to circulatory collapse, cardiovascular complications or even renal problems have also been reported [157]. Although liraglutide and exenatide treatments have been claimed to cause acute pancreatitis in humans [158–161], no evidence of pancreatitis was observed when three different animal species including mice, rats or monkeys were injected with liraglutide at a dose 60 times higher than what was recommended for humans [162]. Furthermore, 13 weeks of exenatide treatment in Zucker diabetic fatty rats, a rat model of T2DM, revealed no evidence of pancreatitis or alteration of pancreatic exocrine cell structure and function [163]. On the contrary, exenatide treatment, instead of evoking pancreatitis, attenuated chemically induced pancreatitis in control and diabetic rodents [164]. Thus, examination of autopsy materials from liraglutide-treated or exenatide-treated patients with T2DM has been advised to settle this dispute [165]. Despite this, FDA required addition of warnings about acute pancreatitis risk for the entire class of incretin-based therapies to drug labels.

The fact that sitagliptin or exenatide treatment was linked to an increased risk of developing acute pancreatitis requiring hospitalization further heated this debate [166]. The American Association of Clinical Endocrinologists, the American Diabetes Association and The Endocrine Society commented on this particular study conducted by Singh and his colleagues, stating that the study was rather a retrospective study, not a prospective one, and did not provide any concrete evidence of pancreatic disease. Thus, no change to the current treatment protocols of people with diabetes was recommended. Nonetheless, the results of nine ongoing, prospective, controlled trials of GLP-1-based therapy with over 65 000 subjects are expected to be available soon and only then will facts about the possible link between incretin-based therapies and acute
pancreatitis be revealed. Although it was suggested that patients should be made aware of the potential side effect of the incretin treatment, it is necessary to keep in mind that diabetes itself is associated with a twofold increase in the incidence of acute pancreatitis. The Committee for the Medicinal Products for Human Use of the EMEA issued a statement in July of 2013 recommending that currently available data did not support an increased risk of pancreatic adverse events concerning incretin therapies. Current labelling of incretin-based therapies includes warnings about the use in patients with a history of pancreatic disease and recommendations to discontinue the treatment in patients who develop pancreatitis.

Examination of the US FDA’s reported adverse event database indicated that pancreatic cancer was more commonly reported among patients who took sitagliptin or exenatide as compared with those who were subjected to other therapies [167]. In reality, chronic subclinical pancreatitis due to GLP-1-based therapy was reported to result in increased diagnosis of pancreatic cancer [168]. These concerns have been validated by other studies suggesting that exendin-4-mediated prolonged GLP-1R activation might result in the proliferation of pancreatic duct glands (PDGs) in rats and enhanced the formation of dysplastic lesions [low-grade murine pancreatic intraepithelial neoplasia (mPanIN)] along with chronic pancreatitis in the Kras<sup>G12D</sup> mouse model [169]. In this particular study, the animals were predisposed to dysplasia, and cross-sectioning of the entire pancreas including longitudinal sections through the main pancreatic duct was necessary to observe any GLP-1-induced changes in PDGs. Therefore, failure to observe overt pancreatitis or the absence of tumours in lean non-diabetic animals treated with exendin-4 in previous studies might be due to animals not being predisposed to dysplasia as well as methodical analysis used to document changes in pancreas [164,170,171]. It is not difficult to imagine that PDGs, in the setting of chronic pancreatitis, could easily be transformed into pancreatic intraepithelial neoplasia-like lesions [165,172]. Although exendin-4 treatment resulted in mPanIN development, the duration of the drug treatment was not sufficient to cause pancreatic cancer in genetically engineered mice [173].

Accordingly, Butler and his colleagues have recently reported a marked expansion of exocrine and endocrine pancreas along with exocrine pancreas dysplasia in patients treated with incretin therapy [174]. This autopsy study reporting abnormal pancreatic findings from T2DM patients treated with sitagliptin or exenatide, instead of settling the dispute, further fuelled the controversy, suggesting that incretin therapy might be associated with a potential risk of pancreatic cancer. On the other hand, significant concerns were raised against Dr Butler’s autopsy study about the number of pancreas samples examined being very small (from seven sitagliptin-treated patients and one exenatide-treated patient) and the statistical method performed not being satisfactory to establish a causal relationship between incretin treatment and pancreatic cancer [175]. Several other investigators also disputed the methodological analysis used in the design of the study [176].

Because of this controversy, on 12–13 June 2013, the National Institute of Diabetes, Digestive and Kidney Diseases in association with the National Cancer Institute (NIDDK-NCI) held a workshop on pancreatitis, diabetes and pancreatic cancer in Lister Hill Auditorium of the NIH Campus, Bethesda, MD. One of the purposes of the gathering (out of many) was to review the effects of anti-diabetic therapy on the development of pancreatic ductal adenocarcinoma (PDAC). Epidemiologic studies revealed that diabetes itself is associated with an 82% increased risk of pancreatic cancer, independent of therapy. Consequently, the increase in the prevalence of T2DM is somehow connected to the increase in the incidence of PDAC. In addition, cohort studies suggested that 1–2% of new-onset adult diabetes mellitus patients would develop PDAC. In this NIDDK-NCI panel, there were a number of questions raised about Dr Butler’s autopsy data regarding precancerous lesions in the pancreases of eight organ donors who had been taking incretin-based drugs. One of the biggest concerns was the big difference in age between the patients with pancreatic cancer and the controls. The patients with pancreatic cancer were in their 50s, whereas the control group was in their 30s. In addition, the type of lesion that Dr Butler reported in the autopsy study was the alpha cell hyperplasia, which is not a cancer (glucagonoma). Moreover, the mass of the cells used to assess hyperplasia in Dr Butler’s study was based on pancreas weight, which is known to change with patient’s age and diabetic status. Lastly, but not least, Dr Butler’s work suggested that the GLP-1R was expressed on the ductal epithelia of the pancreas and in pancreatic cancer. However, the presentation of Alan Moses, Novo Nordisk’s global chief medical officer, revealed no expression of this type of receptor on the ductal epithelia and in pancreatic cancer. The fact that Dr Butler’s findings either came from genetically manipulated animal models or a very restricted set of human autopsy data raised substantial concerns about the interpretation of his data and outcome of his research findings. Physicians, academicians and scientists from pharmaceutical industry in the NIDDK-NCI panel, after having listened to other investigators, all agreed upon the fact that the latest data did not support increased risk for pancreatic cancer associated with use of incretin-based therapies for T2DM. Butler and his colleagues also acknowledged some of these criticisms including the scale of the study rather being small compared with a randomized clinical trial. In a
response letter to various criticisms from different investigators, Butler and his colleagues stated that their effort represented the first evaluation of human pancreas following GLP-1-based therapy and the evaluation of a larger number of human pancreases was essential to reach definitive conclusions especially considering the widespread use of incretin-based therapies [177]. On 28 June 2013, The American Diabetes Association, the European Association for the Study of Diabetes and the International Diabetes Federation issued a joint statement declaring that no alteration of current treatment recommendations was necessary concerning the use of incretin therapy and pancreatic disease for patients with diabetes. Accordingly, the EMEA also issued a similar statement on 26 July 2013.

In addition, some concerns were also raised about GLP-1R activation inducing C-cell hyperplasia of thyroid glands. Animal safety studies conducted on rodents suggested that liraglutide or exenatide treatment might cause C-cell adenocarcinoma [178]. Upon long-term exposure, constant stimulation of GLP-1R induced C-cell proliferation leading to the formation of C-cell adenomas and medullary thyroid carcinomas (MTC) in mice and rats [179]. Hypothetically, long-term exposure to GLP-1R agonists might also induce C-cell neoplasia in human thyroid glands. Thyroid cancer is a rare disease originating from either follicular or parafollicular thyroid cells [180]. Among the thyroid cancers, 75–85% of cases are papillary thyroid carcinoma, and 10–20% of cases are follicular thyroid cancer. MTC only constitutes 5–8% of cases. Despite C-cell neoplasia and especially MTC being very rare in humans, rodents such as mice and rats spontaneously develop C-cell abnormalities ranging from C-cell hyperplasia to C-cell adenoma and MTC. Not surprisingly, daily injection of liraglutide enhanced C-cell abnormalities, generating C-cell carcinomas in both mice and rats [156]. As demonstrated with cell lines in vitro, GLP-1R agonists (exenatide or liraglutide) stimulated rodent thyroid C cells, causing calcitonin release and C-cell proliferation [179]. On the other hand, cell lines established from human C cells could not be stimulated to release calcitonin even if they were exposed to a very high concentration of GLP-1, exenatide or liraglutide. In addition, animal experiments conducted with primates suggested no increase in C-cell proliferation and calcitonin release after the long-term liraglutide treatment [179]. Testing of human C cells in long-term clinical trials also supported these findings. For example, liraglutide stimulation of C-cell proliferation has not been reported in a calcitonin screening study involving 5000 patients [181]. As a result, clinically effective doses of GLP-1R agonists did not increase calcitonin concentrations even if patients were exposed to long-term incretin treatments. The presence of high levels of GLP-1R on thyroid C cells in rodents and absence or low levels of GLP-1R expression on human and/or cynomolgus monkeys’ C cells could account for the differences observed between species [179,182]. Thus, there appeared to be species-specific differences in GLP-1R expression in thyroid. To determine whether C cells in human MTC, C-cell hyperplasia and normal human thyroid express the GLP-1R, immunofluorescence analysis was performed on thyroid tissue samples with MTC (n = 12), C-cell hyperplasia (n = 9), papillary thyroid carcinoma (n = 17) and normal human thyroid (n = 15). The GLP-1R expression was detected on neoplastic and hyperplastic lesions of thyroid C cells in humans. In addition, 18% papillary thyroid carcinomas and C cells and 33% of control thyroid lobes were positive for GLP-1R expression. The presence of GLP-1R expression on human C cells, in some follicular cells and in papillary thyroid carcinomas, suggested that GLP-1 might influence the proliferation rate of other thyroid cancer types as well. A very sensitive radioligand assay involving GLP-1R autoradiography was performed to analyse GLP-1R expression in rodent versus human thyroid [183]. Although increased expression of GLP-1R was detected in rat C-cell hyperplasia and MTC, no GLP-1R expression was detected in normal human thyroid. In other words, although a considerable amount of GLP-1R expression was detected in non-neoplastic and neoplastic C cells in rodents, they were rarely detectable in human C-cell neoplasia. Nevertheless, according to the US FDA adverse event reporting system database, there is indeed an increased risk for thyroid cancer associated with exenatide. Consequently, it is essential to carefully follow up diabetic patients exposed to long-term GLP-1 analogues for any incidence of thyroid cancer [184].

In addition, beta cell growth-promoting properties of incretins have not been replicated in human studies as demonstrated in animal models. The dose of GLP-1, which is used to promote beta cell growth in rodents, typically ranges between 50 and 100 μg/kg of body weight [39,185]. Considering the tolerable dose of GLP-1 in humans is <2 μg/kg body weight [186] and GLP-1 mimetic exenatide (Byetta) is currently prescribed at a dose of 5 μg (injected twice daily) to treat T2DM patients, systemic injections may not be the route of drug delivery to achieve beta cell growth-promoting actions of GLP-1 mimetics in diabetic patients.

Contrary to GLP-1 mimetics, DPP-4 inhibitors have a neutral effect on body weight [187]. This is an intriguing finding because some weight gain would be expected as a result of glucosuria resulting in glucose retention (as fat) in the body. Because DPP-4 has a wide range of substrates such as chemokines, hormones and neuropeptides, side effects and the outcome of long-term DPP-4 inhibition are not known [188]. Although preclinical and clinical trial data concerning sitagliptin or vildagliptin treatments did not indicate an increased risk of pancreatitis in
patients with T2DM [170], common side effects included headache, nasopharyngitis, upper respiratory infections, urinary system infections [189], severe allergic reactions [190] and hypoglycaemia [191].

Conclusions

The first incretin mimetic (exenatide) was approved by the US FDA in April 2005. Soon after that, in October 2006, the US FDA approved the first oral incretin effect amplifier, DPP-4 inhibitor (sitagliptin). So far, several other incretin mimetics have reached the market, and there are even more incretin-based drugs under development awaiting marketing approval. Clinical trials of incretin-based therapies demonstrated that incretins are as effective as other anti-diabetic drugs (sulfonylureas, thiazolidinediones and long-acting insulin therapies), if not superior for improving blood glucose control and achieving weight loss. Although some concerns were raised against incretin-based therapies regarding pancreatitis or pancreatic cancer, the US FDA review of preclinical and some limited clinical data from all currently available incretin therapies revealed no concern for pancreatic disease. The fact that no clinical incretin-based treatment study has been suspended for safety concerns further supported this notion. Consequently, regulatory agencies advised no change to current treatment protocols of patients with diabetes treated with incretin-based therapies. The fact that 80 000 subjects are currently enrolled in ongoing cardiovascular disease (CVD) outcome trials required by the US FDA, the long-term effects of incretin-based therapies concerning cardiovascular morbidity and mortality will hopefully be available soon in patients with T2DM. Among CVD outcome trials, specifically LEADER (liraglutide), EXSCEL (exenatide once-weekly), ELIXA (lixisenatide) and REWIND (dulaglutide) are expected to be completed between 2016 and 2019.

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Conflict of interest

The authors declare that there is no duality of interest associated with this manuscript.

References

Incretin Therapy for Diabetes


Incretin Therapy for Diabetes


128. McClane PL, Irwin N, Cassidy RS, Holst JJ, Gauld VA, Flatt PR. GLP receptor antagonist reverses obesity, insulin resistance, and associated metabolic disturbances induced in mice by prolonged consumption of high-fat


