The incretin system and cardiometabolic disease

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Rates of type 2 diabetes, obesity and their associated detrimental cardiovascular effects are rapidly increasing. Despite the availability of several treatment options for type 2 diabetes and the use of intensive regimens combining several antidiabetic drugs, less than one-half of all patients reach a target glycosylated hemoglobin level of less than 7%. Disease progression due to ongoing deterioration of pancreatic islet cell health and beta-cell function is likely responsible. Therefore, there is a need to identify new pharmacological compounds that may not only treat hyperglycemia, but may also correct impaired glucose homeostasis and preserve endogenous beta-cell function. Identification and characterization of the incretin system and its effect on glucose homeostasis have resulted in the development of new antidiabetic agents that target these concerns. The current review examines the incretin effect and the pharmacological agents that have been developed based on the understanding of this physiological system. The influence of incretins on the cardiovascular system beyond the proatherogenic effect of type 2 diabetes will also be discussed.

Key Words: *Cardiometabolic syndrome; Diabetes; Dipeptidyl peptidase-4; Incretin system*

Globally, the rates of type 2 diabetes and its associated detrimental cardiovascular effects are rapidly increasing (1). Likewise, the obesity epidemic continues to represent a global health care issue, with an estimated 1.6 billion adults overweight worldwide (2). These ever-increasing rates of obesity, particularly abdominal obesity, will only fuel the number of individuals with insulin resistance and the cardiometabolic syndrome, placing even more individuals at particular risk for cardiovascular disease and type 2 diabetes. Despite the availability of several treatment options for type 2 diabetes and the use of intensive regimens combining several antidiabetic drugs, less than one-half of all patients reach a target glycosylated hemoglobin (HbA1c) level of less than 7% (3). This may reflect the ineffective implementation of current pharmacotherapies, but may also be secondary to disease progression due to ongoing deterioration of pancreatic islet cell health and beta (β)-cell function (4). Therefore, there is a need to identify new pharmacological compounds that may not only treat hyperglycemia but may also correct impaired glucose homeostasis and preserve endogenous β -cell function, because patients with newly diagnosed diabetes have only approximately 50% normal β -cell function, with further progressive loss over time (5). Identification and characterization of the incretin system and its effect on glucose homeostasis has resulted in the development of new antidiabetic agents that not only improve glycemic control but may also preserve β -cell function. The current review examines the incretin effect and the pharmacological agents that have been developed based on the understanding of this physiological system. The influence of incretins on the cardiovascular system beyond the proatherogenic effect of type 2 diabetes will also be described in depth.

WHAT IS THE INCRETIN SYSTEM?

The regulation of plasma glucose depends on signals from the brain, the nervous system and the gastrointestinal tract. These signals

Le système des incrétines et la maladie cardiométabolique

On assiste à une augmentation rapide des taux de diabète de type 2 et d'obésité, ainsi que de leurs effets cardiovasculaires néfastes. Malgré les nombreuses options thérapeutiques offertes pour lutter contre le diabète de type 2 et l'utilisation de schémas intensifs alliant plusieurs agents antidiabétiques, moins de la moitié de tous les patients atteignent le taux d'hémoglobine glyquée ciblé, soit moins de 7 %. La maladie progresse probablement en raison d'une détérioration graduelle des cellules des îlots de Langerhans et du dysfonctionnement des cellules bêta. C'est pourquoi il faut tenter de mettre au point de nouveaux agents pharmacologiques qui, non seulement traiteraient l'hyperglycémie, mais corrigeraient aussi l'homéostasie anormale du glucose tout en préservant le fonctionnement des cellules bêta endogènes. La reconnaissance et la caractérisation du système des incrétines et de ses effets sur l'homéostasie du glucose ont mené au développement de nouveaux antidiabétiques qui ciblent ces aspects. La présente synthèse analyse l'effet des incrétines et des agents pharmacologiques mis au point grâce à l'approfondissement des connaissances sur ce système physiologique. On abordera en outre l'influence des incrétines sur l'appareil cardiovasculaire, au-delà de l'effet proathérogène du diabète de type 2.

influence the secretion of pancreatic hormones, namely insulin and glucagon. Hyperglycemia is largely a result of defects in both pancreatic islet alpha (α)- and β -cell function, leading to decreased insulin secretion and elevated glucagon release from the endocrine pancreas (6). The observation that an oral glucose load resulted in greater insulin release than an equal amount of glucose administered intravenously suggested that glucose transit through the gut stimulated the release of insulinotropic hormones (7). This phenomenon, by which insulin secretion is augmented by oral glucose intake by intestinally derived peptides, as opposed to an intravenous glucose load, has been referred to as the incretin effect (8). The incretin effect is believed to account for 50% to 70% of the total amount of insulin secreted after oral glucose consumption (9), an effect that appears to be attenuated in patients with type 2 diabetes who have a defective response to glucose stimulation characterized by diminished insulin secretion and impaired insulin action.

The incretin system thus serves as a potential therapeutic target for the management of diabetes because the incretin effect is absent or substantially diminished in this disease state. Insulin secretion in healthy volunteers tends to double in response to an oral versus an intravenous glucose load, largely due to the incretin effect, whereas type 2 diabetic patients display only an approximate 20% increase in insulin secretion with oral versus intravenous glucose (8). This largely stems from the observation that individuals with type 2 diabetes or impaired glucose tolerance exhibit reduced postprandial release of glucagon-like polypeptide-1 (GLP-1), which then leads to both decreased insulin secretion (8) and inadequate glucagon suppression (10).

Two peptides – glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 – are primarily responsible for the incretin effect. Both hormones are secreted by the small bowel and are rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4), which

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preferentially cleaves peptides with an alanine or proline residue in the second aminoterminal position (11). DPP-4 is a cell-surface serine protease widely expressed in many tissues including the liver, intestinal brush border membranes, lymphocytes and endothelial cells (12). It displays enzymatic activity in both its membrane-anchored and cleaved or circulating forms. Both incretin mimetics and DPP-4 inhibitors form the basis for the current incretin-based therapy.

GIP

The first incretin hormone identified, GIP, was initially isolated from porcine intestinal extracts and given the name 'gastric inhibitory polypeptide' for its weak inhibitory effect on gastric acid secretion (13). Subsequent investigations revealed that at physiological levels, GIP had a greater effect on glucose-stimulated insulin secretion in both animals and humans, and thus, it was renamed to reflect this action (14). GIP is a 42-amino acid peptide that is synthesized in duodenal and jejunal enteroendocrine K-cells, primarily in response to fat or glucose ingestion. It is highly conserved across species, with over 90% of the human GIP amino acid sequence being identical to rat, mouse, porcine and bovine GIP (15). GIP acts through the GIP receptor (GIPR), which is expressed not only in the pancreas, but also throughout the gastrointestinal tract (including the stomach and small intestine), in adipose tissue, in the heart and vascular endothelium, and in the brain (16). GIPR belongs to the seven-transmembrane domain, heterotrimeric G protein-coupled glucagon receptor superfamily (17), and its activation results in increased intracellular calcium and cyclic AMP levels, as well as the activation of the phosphoinositide 3-kinase (PI3K), protein kinase (PK) A, PKB and mitogen-activated protein kinase (MAPK) pathways (9). Biologically active GIP has a half-life of approximately 7 min in healthy human patients and 5 min in individuals with type 2 diabetes (18). As mentioned previously, it is enzymatically inactivated by DPP-4 and cleared through the kidney.

Via direct effects on pancreatic β -cells, GIP causes glucose-dependent insulin secretion. It promotes β -cell exocytosis by increasing intracellular cyclic AMP. It stimulates insulin biosynthesis and, through its ability to activate PKA-dependent, MAPK-dependent and PI3K-dependent pathways, it exhibits growth factor-like properties, stimulating β -cell proliferation and improving β -cell survival (19,20). The elimination of GIP action in animal models – either by treatment with GIPR antagonists or GIP peptide antagonists, or via the development of GIPR-deficient mice – supports its role as an essential incretin hormone because its loss results in reduced postprandial insulin release, mild glucose intolerance in response to oral glucose administration and decreased glucose-dependent insulin secretion (21,22). In healthy human subjects, GIP serves as a potent incretin; however, in type 2 diabetic patients, most of the glucoregulatory actions of GIP appear to be diminished (23,24). Furthermore, GIP in individuals with liver cirrhosis, basal hyperglucagonemia or euglycemia results in the stimulation of glucagon secretion rather than its suppression, leading to an increase in glucose levels (25,26). Thus, even though GIP demonstrates a glucose-lowering effect, although diminished in diabetic patients, its inability to suppress glucagon secretion further makes it a less than ideal target for pharmacotherapy in the treatment of type 2 diabetes.

Aside from effects on pancreatic β -cell physiology, GIP appears to influence adipocyte biology as well. Fat ingestion stimulates GIP secretion in humans and GIP plasma levels appear to be increased in some obese individuals (27). GIP may promote lipogenesis by stimulating fatty acid synthesis, upregulating lipoprotein lipase expression, enhancing fatty acid incorporation into triglycerides and reducing glycogen-stimulated lipolysis, yielding a net anabolic fat effect (9). The elimination of GIP activity as observed in GIPR knockout mice results in the preferential use of fat for energy, resistance to diet-induced obesity and an overall reduction in adipocyte mass compared with controls (28). These favourable changes to adipocyte biology translated into improved insulin sensitivity. Similarly, the administration of the GIPR antagonist (Pro³)GIP on a daily basis to genetically obese mice enhanced insulin sensitivity, improved glucose tolerance and reduced

weight gain independent of changes in food consumption (29). These observations suggest that GIP, at least in the obese state, may contribute to further adiposity and insulin insensitivity. This, in turn, may ablate the positive effects on β -cell physiology such as improved insulin secretion, a response that already appears to be diminished in patients with type 2 diabetes. The mechanisms surrounding these antagonistic observations in the setting of obesity remain unclear, although GIP may play a role in switching from fat oxidation to fat accumulation in states in which insulin action is diminished (30), a physiological state commonly seen in individuals with the cardiometabolic syndrome. Recently, the adipokine resistin appeared to be the mediator responsible for the activation of lipoprotein lipase by GIP (31). Resistin, secreted by adipose tissue, is believed to play a key role in the development of insulin resistance, and the promotion of endothelial activation and dysfunction, which serve as the initiating steps in atherogenesis (32). Furthermore, the early administration of (Pro³)GIP in genetically obese mice appeared to prevent the development of diabetes and many of the metabolic abnormalities associated with obesity (33). Thus, whether the administration of GIP and GIPR activation, or the blockade of GIPR signalling would serve as the more effective therapeutic intervention in individuals with type 2 diabetes remains to be determined.

GLP-1

The second incretin hormone, GLP-1, is primarily made in the enteroendocrine L-cells in the distal ileum and colon (9). L-cells are intestinal epithelial endocrine cells that contact both luminal nutrients, on the apical side, and vascular and neural tissues, on the basolateral surface. GLP-1 is one of the products generated from the post-translational processing of proglucagon. In the fasted state, plasma levels are low, ranging in concentration from 5 pmol/L to 10 pmol/L. However, plasma levels increase within minutes of eating, reaching circulating concentrations of 15 pmol/L to 50 pmol/L (11). The oral ingestion of fats and carbohydrates serves as the primary physiological stimulus for GLP-1 secretion. Two forms of GLP-1 are present in the circulation, GLP-1₇₋₃₇ amide and GLP-1₇₋₃₆ amide. Both forms have equal potency, although the GLP-1₇₋₃₆ amide is more abundant in humans following meal ingestion (34). Secretion of GLP-1 appears to be biphasic, with an early phase within 15 min of food consumption and a delayed, longer second phase 30 min to 60 min following ingestion (35). The circulating half-life of GLP-1 is less than 2 min, being degraded by DPP-4 and cleared by the kidney (36). In patients with obesity or type 2 diabetes, the postprandial levels of intact GLP-1 are significantly reduced compared with normal subjects (37), likely secondary to reductions in GLP-1 secretion because the elimination rates of GLP-1 are similar in healthy, obese and diabetic individuals (38).

GLP-1 mediates its physiological effects via the GLP-1 receptor (GLP-1R). GLP-1R is expressed in a wide range of tissues, including α - and β -pancreatic islet cells, and the lung, heart, kidney, stomach, intestine, pituitary gland and hypothalamus (9). It belongs to the class B family of seven-transmembrane-spanning, G-protein-coupled receptors, which also includes the receptors for glucagon and GIP (17). Stimulation of GLP-1R leads to increases in intracellular calcium levels, adenylate cyclase activity, and the activation of the PKA, PKC, PI3K and MAPK signalling pathways (9).

GLP-1 exerts a number of actions that are important in maintaining glucose homeostasis. GLP-1 does not only act on pancreatic β -cells to stimulate insulin secretion, but at the same time, acts synergistically with glucose to replenish insulin stores by promoting insulin gene transcription and biosynthesis (39). GLP-1 improves β -cell glucose sensitivity by upregulating the expression of glucose transporters and glucokinases, potentially being able to restore glucose responsiveness in resistant β -cells (40). GLP-1R activation enhances β -cell mass by stimulating β -cell proliferation and neogenesis, while simultaneously inhibiting apoptosis (41,42). In addition to these favourable effects on insulin secretion and β -cell survival, GLP-1 inhibits glucagon secretion and stimulates somatostatin release, measures that further

counteract hyperglycemia. The glucagonostic effect of GLP-1 appears to be glucose dependent, like insulin secretion, and thus, the likelihood of becoming hypoglycemic is diminished (43). The physiological effects of endogenous GLP-1 have been demonstrated using GLP-1R antagonists and GLP-1R knockout mice. Eliminating GLP-1 activity via treatment with GLP-1R antagonists resulted in elevated fasting glucose and glucagon levels, impaired glucose tolerance and decreased glucose-stimulated insulin levels (44,45). GLP-1R knockout mice have mild fasting hyperglycemia and a moderate degree of glucose intolerance following oral glucose challenge, likely secondary to impaired glucose-stimulated insulin release (46). These mice also demonstrate abnormal pancreatic islet cell development and impaired β -cell regeneration.

GLP-1 acts on the central nervous system to influence feeding behaviour. In rodents, the administration of GLP-1R agonists inhibits short-term food and water intake, and decreases body weight (47,48). A similar effect is seen in human subjects. Healthy, diabetic and obese individuals treated with GLP-1R agonists displayed early satiety, decreased oral intake and a subsequent loss in weight (11). Direct GLP-1-mediated effects on the gastrointestinal tract lead to delayed gastric emptying, which contributes to reductions in postprandial plasma glucose levels (49) and triglyceride levels (50). Thus, by lowering both glucose and lipid concentrations, GLP-1 may reduce components of the cardiometabolic syndrome in patients with type 2 diabetes. In recent-onset type 1 diabetes, where some endogenous insulin secretion in response to meals still exists, the infusion of GLP-1 resulted in a substantial reduction in blood glucose levels following meals. This was believed to be secondary to GLP-1-mediated suppression of glucagon secretion and human pancreatic polypeptide release, and the inhibition of gastric emptying (51). Thus, GLP-1 glycemic effects in type 1 diabetes cannot solely be accounted for by interactions with pancreatic β -cells and combination therapy with insulin, and long-acting GLP-1 agonists may provide enhanced diabetes control. Further studies with combination therapy are required.

DPP-4

Originally identified as the lymphocyte cell surface marker CD26, DPP-4 is a 766-amino acid, membrane-anchored, serine protease that preferentially cleaves a large number of chemokines with an alanine or proline residue at the second aminoterminal position (52). It is a member of a large enzyme family that cleaves structurally related peptides. Most of DPP-4 is extracellular, with a very short six-amino acid intracellular sequence. Enzymatic activity is exhibited by both the membrane-bound and soluble forms. Both native GLP-1 and GIP are rapidly cleaved by DPP-4 at the position 2 alanine. DPP-4 acts on GIP₁₋₄₂ to generate GIP₃₋₄₂, which has no known biological activity. DPP-4 cleavage of the native GLP-1₇₋₃₆ amide yields GLP-1₉₋₃₆, which appears to also demonstrate insulin-independent glucose clearance and cardioprotective effects, as discussed below (53).

The physiological role of DPP-4 in regulating incretin biology is derived from studies conducted in rodents with either DPP-4 gene inactivation or DPP-4-specific inhibition. A strain of Fischer 344 rats that harbour a mutation in the DPP-4 gene, which results in the synthesis of a mutant protein that is rapidly degraded before becoming active, exhibits increased levels of plasma GLP-1, increased insulin levels following oral glucose intake and overall improved glucose tolerance compared with controls (54). DPP-4 knockout mice also display improved glucose tolerance, which is associated with increased levels of GIP and GLP-1, improved insulin secretion after oral glucose challenge, resistance to diet-induced obesity, reduced fat accumulation and reduced food intake despite increased energy expenditure (55,56). Similarly, rodents with experimental diabetes showed improvements in glucose tolerance when treated with DPP-4 inhibitors. The DPP-4 inhibitor des-fluoro-sitagliptin significantly reduced blood glucose and HbA1c levels in diabetic mice (57). These mice also exhibited improvement in pancreatic insulin content and increased numbers of insulin-positive β -cells, likely secondary to enhanced GLP-1

antiapoptotic and proliferative effects on β -cells. Furthermore, inhibition of DPP-4 with sitagliptin was demonstrated to prolong islet graft survival in streptozotocin-induced diabetic mice and maintain glycemic control compared with control mice (58).

In human subjects, levels of DPP-4 activity appear to be higher in individuals with chronic hyperglycemia and type 2 diabetes (59,60). Type 2 diabetic patients with poor glycemic control as assessed by an HbA1c level of greater than 8.5%, showed significantly higher DPP-4 activity than newly diagnosed diabetic patients or subjects with impaired glucose tolerance (59). Furthermore, in type 2 diabetic patients, DPP-4 activity was positively correlated with fasting plasma glucose and HbA1c, suggesting that individuals with worse glycemic control had higher rates of DPP-4 activity (60). Thus, increased DPP-4 activity may result in decreased levels of GLP-1 and GIP, contributing to impaired postprandial glucose control. However, whether elevated circulating DPP-4 activity leads to reduced levels and activity of GLP-1 or GIP in human subjects remains unknown. Nevertheless, treatment with DPP-4 inhibitors in humans with type 2 diabetes does appear to improve either fasting glucose or glucose tolerance in association with improved β -cell function and reduced plasma glucagon levels (61).

As alluded to earlier, DPP-4 has several peptide substrates in addition to GLP-1 and GIP, and may therefore participate in physiological roles outside of the incretin system. Among the physiological substrates for DPP-4 are stromal cell-derived factor (SDF)-1- α and - β . Levels of the proinflammatory chemokine SDF-1- α are significantly increased in DPP-4 knockout mice (62). In a model of antigen-induced arthritis in wild-type mice, plasma DPP-4 activity was noted to be reduced with the induction of joint inflammation. When DPP-4 knockout mice were exposed to the same antigen, the severity of the arthritis was increased, coupled by elevated SDF-1- α levels and increased numbers of SDF-1 receptor-positive cells infiltrating the joint space (62). DPP-4 knockout mice also demonstrate alterations in their immune cell and cytokine profile compared with wild-type controls after immunization (63). DPP-4-deficient mice have significantly fewer CD4⁺ natural killer cells in their peripheral blood and, after immunization with pokeweed mitogen, serum levels of total immunoglobulin (Ig) G, IgG1, IgG2a and IgE, in addition to levels of interleukin-2 and interleukin-4, are reduced. The significance of these effects of DPP-4 on immune function in type 2 diabetic human subjects treated with DPP-4 inhibitors remains unclear, although a recent meta-analysis (64) suggested that DPP-4 inhibitors may increase the risk of urinary tract infections and nasopharyngitis. Furthermore, the biologically active form of brain natriuretic peptide-1-32, a peptide whose secretion by myocytes is stimulated by stretch and volume overload, also appears to serve as a substrate for DPP-4. Inhibition of DPP-4 impairs brain natriuretic peptide cleavage, although the physiological effects of this remain to be elucidated (65).

INCRETIN-BASED THERAPIES FOR TYPE 2 DIABETES

The understanding of the incretin effect on the pathophysiology of type 2 diabetes, and the pleiotropic actions of GLP-1 and GIP on glycemic control, has led to the development of incretin-based therapies for diabetes. The incretin effect appears to be substantially reduced in type 2 diabetes, a disorder with reductions in meal-stimulated levels of GLP-1 and increased levels of DPP-4 activity (8,10,59). Several of the metabolic derangements present in type 2 diabetes, including defective glucose-stimulated insulin secretion, slow insulin secretory response to meals, hyperglucagonemia, reduced pancreatic β -cell insulin content and reduced pancreatic β -cell mass may be addressed using incretin-based therapies (11). Furthermore, the incretin effect plays a significant role in controlling post-meal hyperglycemia, which makes a significant contribution to HbA1c (66), independently predicts diabetes-related complications (67), especially macrovascular disease, and if reduced, may lead to cardiovascular risk reduction (68). Intravenous infusions of GLP-1, resulting in concentrations of biologically active GLP-1 from 10 pmol/L to 20 pmol/L, lowered blood glucose in type 2 diabetic patients via the suppression of glucagon secretion and the transient

glucose-stimulated secretion of insulin (69,70). Continuous subcutaneous GLP-1 infusion in type 2 diabetic patients over a six-week period, which achieved plasma GLP-1 levels of 60 pmol/L to 70 pmol/L, resulted in improved fasting and 8 h mean plasma glucose concentrations (decreased by 4.3 mmol/L and 5.5 mmol/L, respectively; $P < 0.0001$), a reduction in HbA1c by 1.3% ($P = 0.003$) and an average decrease in body weight by 1.9 kg, compared with continuous subcutaneous saline administration (71). However, continuous intravenous or subcutaneous infusions of GLP-1 are not practical in the outpatient setting, and native GLP-1 is rapidly inactivated. Therefore, pharmacological efforts directed toward promoting the actions of GLP-1 have focused on sustained activation of the GLP-1R via the use of injectable degradation-resistant GLP-1 analogues or by inhibiting GLP-1 inactivation via selective DPP-4 inhibition.

Incretin mimetics – GLP-1 analogues

Exenatide (Byetta, Amylin Pharmaceuticals Inc/Eli Lilly and Co, USA) – synthetic exendin-4 – was the first GLP-1 analogue or GLP-1R agonist approved by the United States Food and Drug Administration (FDA) in 2005 for its use as adjunctive therapy in patients with type 2 diabetes. Exendin-4 is a GLP-1-related peptide isolated from the venom of the *Heloderma suspectum* lizard (72). It has approximately 53% amino acid sequence homology to mammalian GLP-1, is a potent mammalian GLP-1R agonist and is resistant to degradation by DPP-4 (73). The synthetic form, exenatide, is administered subcutaneously. It has a circulating half-life of 60 min to 90 min, with concentrations remaining elevated up to 6 h after a single injection (74). Clinical trials assessing the efficacy of adding exenatide (twice daily subcutaneous injections of 5 µg or 10 µg) to ongoing oral antidiabetic agents (metformin, sulfonylureas and/or thiazolidinediones) in type 2 diabetic patients with suboptimal glycemic control demonstrated that exenatide therapy substantially improved several glycemic and nonglycemic outcomes versus placebo (75-78). Patients receiving exenatide were more likely to achieve an HbA1c value of less than 7% than those receiving placebo (45% versus 10%, respectively; risk ratio 4.2 [95% CI 3.2 to 5.5]), and showed reductions in fasting plasma glucose (–1.5 mmol/L [95% CI –1.83 mmol/L to –1.17 mmol/L]) and postprandial glucose excursions up to 87% compared with baseline (64). In terms of nonglycemic outcomes, exenatide therapy appeared to demonstrate progressive weight loss, although there were no significant changes in lipid profile (64). Exenatide therapy has also been compared with insulin glargine and biphasic insulin aspart in type 2 diabetic patients with suboptimal control on oral agents. In open-label, noninferiority studies, there was no significant difference in HbA1c or fasting plasma glucose levels between exenatide and the insulins tested, although postprandial glycemia was reduced more with exenatide (79,80). Also, compared with insulin therapy, exenatide treatment resulted in a significant degree of weight loss (–4.76 kg [95% CI –6.03 kg to –3.49 kg]) (64). A long-acting release (LAR) formulation of exenatide, administered subcutaneously once weekly for 15 weeks to diabetic subjects suboptimally controlled with metformin and/or diet, reduced HbA1c by a mean ($\pm$ SD) of $-1.7 \pm 0.3\%$ ($P < 0.0001$), reduced fasting plasma glucose by -2.2 ± 0.5 mmol/L ($P < 0.001$) and resulted in a reduction in body weight (-3.8 ± 1.4 kg, $P < 0.05$) compared with placebo (81). Thus, exenatide LAR may offer a once-weekly treatment option for type 2 diabetes.

In terms of adverse events, no significant differences were observed in overall rates of hypoglycemia, although gastrointestinal side effects such as nausea and vomiting were more frequently reported with exenatide therapy; these side effects were reduced with once-weekly exenatide LAR treatment. Recently, there was a label change to reflect the potential increased risk of pancreatitis with exenatide therapy. Also, up to 67% of individuals treated with exenatide developed antibodies to it, although this did not appear to impair its antidiabetic effectiveness (64).

A second GLP-1R agonist, which has been developed and is currently undergoing phase III clinical trials, is liraglutide (Victoza, Novo Nordisk, USA). Liraglutide is a long-acting, DPP-4-resistant GLP-1

analogue that binds noncovalently to albumin and has a half-life of 10 h to 14 h, making it suitable for once-daily injection (82). In phase II studies, liraglutide combined with metformin therapy reduced both fasting and postprandial glucose concentrations, and lowered HbA1c levels by 0.8%, while simultaneously promoting a modest but significant degree of weight loss (83). Early phase III clinical studies as part of the Liraglutide Effect and Action in Diabetes (LEAD) program, although not yet formally published, suggest that liraglutide is well tolerated as add-on therapy to both metformin and rosiglitazone, and results in a greater number of patients reaching an HbA1c value of less than 7% after 26 weeks of therapy.

Incretin enhancers – DPP-4 inhibitors

By inhibiting DPP-4, the goal of therapy is to prevent the physiologically rapid degradation of GLP-1. DPP-4 inhibitors have been developed to specifically inhibit DPP-4 activity, which leads to a rise in, and stabilization of, endogenous postprandial levels of intact GLP-1. Sitagliptin (Januvia, Merck Frosst Canada Ltd) was the first oral selective DPP-4 inhibitor approved by the FDA in October 2006, for use as monotherapy or in combination with metformin or a thiazolidinedione. A second DPP-4 inhibitor, vildagliptin (Glavus, Novartis, USA) is currently awaiting FDA approval, although it has been approved by the European Medicines Agency.

The efficacy of sitagliptin and vildagliptin in terms of glycemic outcomes have not been compared directly, although each has been compared with placebo. Sitagliptin once-daily monotherapy appears to substantially improve glycemic control and fasting plasma glucose (84), and its addition to ongoing metformin therapy in patients with type 2 diabetes led to further reductions in HbA1c, fasting plasma glucose and 2 h post-meal glucose (85). Both agents appear to lower HbA1c to a similar degree, by approximately 0.74% (95% CI –0.85% to –0.62%), with similar efficacy as monotherapy or add-on therapy (64). Compared with currently available therapies, the degree of HbA1c lowering offered by DPP-4 inhibitors appears to be similar or perhaps slightly less than that afforded by other oral agents; however, their true value may lie in preservation of β -cell mass and function. In individual comparisons, sitagliptin was found to be noninferior to glipizide (86), while noninferiority was shown for vildagliptin when compared with thiazolidinediones (87,88) but not when compared with metformin (89). DPP-4 inhibitors reduced fasting plasma glucose (weighted mean difference –1.0 mmol/L [95% CI –1.2 to –0.8]) and postprandial glycemia (64). Aside from the favourable effects on glycemic indexes, in noninferiority trials, sitagliptin had a favourable weight profile compared with glipizide (–2.5 kg versus 1.0 kg) (86) and vildagliptin had a favourable weight profile compared with thiazolidinediones but not compared with metformin (87-89). When all the DPP-4 inhibitor trials that reported data on weight were combined as part of a meta-analysis, a minimal increase in weight compared with placebo (weighted mean difference 0.5 kg [95% CI 0.3 kg to 0.7 kg]) was observed, which is not unexpected, given the improved glycemia (64). In terms of the lipid profile, no consistent changes have been identified with DPP-4 treatment.

The progressive rise in HbA1c typically observed in patients with type 2 diabetes is believed to coincide with further decreases in pancreatic β -cell function. Various markers have been used to estimate β -cell function in humans, including homeostasis model assessment- β , a marker of fasting insulin secretion, and the proinsulin-to-insulin ratio. Sitagliptin monotherapy over a 24-week period significantly improved both of these parameters; however, whether this observed improvement was a drug-specific effect or secondary to improved glycemia and reductions in glucose toxicity is unclear (90,91).

Sitagliptin appears to be well tolerated at doses of 100 mg once daily, while vildagliptin has routinely been administered at a dose of 50 mg twice daily or 100 mg once daily. Despite the large number of potential substrates for DPP-4, including roles in inflammation and the immune response, no characteristic pattern of adverse events has been observed. Rates of hypoglycemia between DPP-4 inhibitors and

the comparator oral agents do not significantly differ (1.6% versus 1.4%, respectively; risk ratio 1.0 [95% CI 0.5 to 1.9]) (64), and the gastrointestinal side effects reported with GLP-1 analogues are not seen. However, an increased risk of urinary tract infections (3.2% for DPP-4 inhibitors versus 2.4% for comparator; risk ratio 1.5 [95% CI 1.0 to 2.2]) and nasopharyngitis (6.4% for DPP-4 inhibitors versus 6.1% for comparator; risk ratio 1.2 [95% CI 1.0 to 1.4]) does suggest a possible increased risk of infection with DPP-4 inhibitors, perhaps secondary to alterations in immune function (64).

As with any new agents, caution is advised because the potential for unexpected consequences exists (92). The majority of the trials completed to date with the GLP-1 analogues and the DPP-4 inhibitors have lasted less than 30 weeks. Continued evaluation with both long-term efficacy and safety trials and postmarketing surveillance will be required to ensure that these new classes of hypoglycemic agents are safe and efficacious. Long-term studies are being performed. The glucose-lowering effects of incretin-based therapies are comparable with existing agents when one corrects for the lesser reductions in HbA1c observed with the baseline HbA1c levels in contemporary trials. The potential extraglycemic benefits offered by these agents may reveal their true benefits and define their role in the treatment of diabetes. For example, GLP-1R agonist therapy results in weight loss. In the case of DPP-4 inhibitors, no obvious extraglycemic benefits have been observed consistently to date. However, if the preclinical results from in vitro and rodent studies demonstrating GLP-1 analogue and DPP-4 inhibitor-mediated positive effects on β -cell physiology and survival emerge in human patients, incretin therapy may serve to not only enhance glycemic control but to also delay and halt the progression of type 2 diabetes.

INCRETINS AND THE CARDIOVASCULAR SYSTEM

The presence of GLP-1Rs in rodent and human vascular and cardiac tissues suggests a potential role for the incretin system in cardiovascular physiology and disease. Although no large-scale clinical trials using incretin-based therapies have been completed to examine hard cardiovascular end points, several animal studies and small human safety trials have demonstrated that incretins, in particular GLP-1, have a beneficial effect on the cardiovascular system.

Mice lacking GLP-1R have impaired cardiac structure and function, implicating a role for GLP-1 in normal murine cardiac physiology (93). GLP-1R knockout mice at two months of age exhibit reduced resting heart rate and elevated left ventricular (LV) end-diastolic pressures compared with wild-type controls. As these GLP-1R-deficient mice age, histological and echocardiographic analyses reveal increased septal and posterolateral myocardial wall thickness, impaired LV contractility and diastolic dysfunction. Furthermore, either the administration of GLP-1 or GLP-1R agonists in rats results in an increase in arterial blood pressure and heart rate via a catecholamine-independent effect (94,95). The potential mechanism of GLP-1 action may reside in the stimulation of GLP-1Rs in the autonomic control centres of the central nervous system, which subsequently leads to the direct activation of the sympathetic nervous system to modify cardiovascular function (95). However, GLP-1R agonists do not appear to significantly increase either heart rate or blood pressure in humans.

An inadequate amount of energy leads to mechanical failure of the heart. The first step in cardiac energy metabolism depends on the cellular uptake of the energy source. In the case of advanced heart failure, insulin resistance develops in the myocardium, resulting in a decline in glucose utilization (96). Therefore, interventions that enhance myocardial glucose uptake may have a favourable effect on cardiac contractility. GLP-1, with its insulinotropic and insulinomimetic properties, appears to protect the failing heart, in part by enhancing myocardial glucose uptake. In a canine model of rapid pacing-induced cardiomyopathy, infusion of recombinant GLP-1 over a 48 h period significantly improved cardiac output, LV hemodynamics and systemic vascular resistance (97). Recombinant GLP-1 appeared to increase

myocardial insulin sensitivity and subsequent myocardial glucose uptake. The same group further demonstrated that the enzymatically cleaved form of GLP-1, GLP-1₉₋₃₆, similarly served as an active peptide in mediating this physiological response (98).

Similar improvements in cardiac function were seen in patients with chronic heart failure (99), patients who underwent coronary artery bypass grafting (100) and individuals with LV dysfunction after successful reperfusion following acute myocardial infarction (101). In 12 patients with New York Heart Association class III or IV heart failure symptoms, a five-week infusion of GLP-1 added to standard therapy was well tolerated. When these patients were compared with matched controls on standard therapy, they demonstrated a significant improvement in LV ejection fraction (LVEF) ($21 \pm 3\%$ to $27 \pm 3\%$, $P < 0.01$), functional status as assessed by 6 min walk distance (232 ± 15 m to 286 ± 12 m, $P < 0.001$) and quality of life as evaluated by the Minnesota Living with Heart Failure quality of life score (64 ± 4 to 44 ± 5 , $P < 0.01$) (99). Among patients with an acute myocardial infarction and an LVEF of less than 40% after successful primary angioplasty, a 72 h infusion of GLP-1 significantly improved LVEF, global wall motion score indexes and regional wall motion score indexes, as assessed by pre- and post-GLP-1 infusion echocardiograms (101). Perioperative administration of GLP-1 in coronary artery bypass graft patients resulted in better glycemic control and similar hemodynamic recovery, with less need for vasoactive and inotropic infusions, compared with patients receiving standard therapy (100).

GLP-1 also appears to exert a protective effect in settings of myocardial ischemia and injury. Using both isolated perfused and intact rat hearts, the infusion of GLP-1 had direct protective effects in terms of decreasing infarct size. This GLP-1-mediated protective effect appeared to involve the activation of the prosurvival PI3K/Akt and the p42/44 MAPK pathways, similar pathways that are activated by GLP-1 in pancreatic cells (102). After transient coronary artery occlusion, GLP-1 appears to enhance recovery of LV function via favourable effects on cardiac energy metabolism (103). In isolated rat hearts subjected to 30 min of low-flow ischemia followed by 30 min of reperfusion, with or without GLP-1, the GLP-1-treated group demonstrated significant improvements in LV end-diastolic pressure (103). These improvements coincided with increasing myocardial glucose uptake, nitric oxide production and the translocation of glucose transporter-1. However, the mechanisms underlying these postischemic, GLP-1 cardioprotective effects may not entirely be mediated through GLP-1R. In Langendorff isolated heart preparations from wild-type and GLP-1R knockout mice exposed to 30 min of global ischemia followed by 40 min of reperfusion, GLP-1₇₋₃₆, GLP-1₉₋₃₆ or exendin-4 pretreatment protected the heart from ischemia-reperfusion injury in both groups and promoted functional recovery to a similar extent, compared with untreated controls (104). GLP-1₇₋₃₆ and GLP-1₉₋₃₆ treatments displayed potent vasodilatory effects by increasing coronary flow rates in isolated hearts, a response that was accompanied by increases in cyclic GMP release. The observed vasodilatory response was blunted with DPP-4 treatment, suggesting a prominent role for GLP-1₉₋₃₆ in mediating this effect via a nitric oxide/cyclic GMP-dependent pathway, independent of the known GLP-1R.

Endothelial dysfunction implies the diminished production or availability of nitric oxide, or an imbalance in the relative contribution of endothelium-derived relaxing and contracting factors. An impaired and activated endothelium predisposes the vessel wall to vasoconstriction, leukocyte adherence, platelet activation, thrombosis and vascular inflammation, and thus, sets the stage for atherosclerosis (105). Endothelial dysfunction is associated with insulin resistance and type 2 diabetes; thus, interventions that improve both these parameters may translate into improved survival. Infusion of GLP-1 in type 2 diabetic patients with stable coronary artery disease, but not in healthy subjects, resulted in a significant improvement in endothelial function as assessed by postischemic flow-mediated dilation of the brachial artery using ultrasonography (106). Furthermore, the beneficial effects of GLP-1 on endothelium-dependent vasodilation in humans appears to be

differentially modulated by various sulphonylureas (107). Glyburide appeared to abolish GLP-1-induced acetylcholine-mediated vasodilation, while glimepiride did not alter this effect; however, the mechanism for this difference was not explored (107).

The modulation of DPP-4 activity not only affects the concentration of GLP-1 and GIP levels but may also influence concentrations of its other substrates, namely SDF-1- α , which influences stem cell biology. Therefore, DPP-4 may play a role in regulating the migration, homing and engraftment of CD34⁺ hematopoietic stem cells. The inhibition of DPP-4 activity was shown to promote human stem cell migration and bone marrow engraftment during cord blood transplantation by enhancing the migratory response of these cells to SDF-1- α (108,109). Levels of intact SDF-1- α were increased with DPP-4 inhibition. A subset of circulating CD34⁺ progenitor cells, endothelial progenitor cells (EPCs), appear to play a significant role in maintaining endothelial health by promoting re-endothelialization and neovascularization in the context of vascular injury and tissue ischemia, respectively (110). The effect of direct DPP-4 activity inhibition on EPC biology has not been directly explored; however, higher plasma concentrations of SDF-1- α have been associated with enhanced levels of EPCs in the circulation (111). In a murine hind limb ischemia model, ischemic stress followed by enalapril treatment resulted in a significant increase in the number of circulating EPCs and the contribution of bone marrow-derived EPCs to areas of ischemia-induced neovascularization (111). This response was associated with a concomitant decrease in total DPP-4 activity in the plasma and a subsequent increase in the concentration of circulating SDF-1- α . Furthermore, SDF-1 regulates adhesion of stem cells in vitro and in vivo, and promotes differentiation of CD34⁺ cells to EPCs (112). In patients with diabetes, the number and function of circulating EPCs is diminished (113). This may explain, in part, why diabetic patients are more susceptible to cardiovascular disease. These same patients also have increased DPP-4 activity. Therefore, it

would be interesting to determine what effect, if any, selective DPP-4 inhibitor treatment with vildagliptin or sitagliptin has on EPCs in this high-risk population.

Ever-increasing rates of obesity will unfortunately result in more patients having insulin resistance, the cardiometabolic syndrome and diabetes. Although societal changes and large-scale public health initiatives will likely be required to decrease this trend, for the time being, new strategies to combat existing disease may provide patients with improved treatment options. Agents that enhance the incretin system, via a physiologically based mechanism of glucoregulation, seem to show promise for the treatment of type 2 diabetes. Although their ability to decrease HbA1c appears to be similar to existing agents, their benefit may lie beyond simple glycemic control. Favourable effects on β -cell function and survival through incretin therapy may translate into a reduction in diabetes disease progression. The cardioprotective properties of GLP-1R agonists may be especially beneficial in patients with type 2 diabetes given their increased risk of cardiovascular morbidity and mortality. Observed improvements in endothelial function and potential benefits to EPC biology may translate into reductions in atherosclerotic disease. Nonetheless, long-term clinical studies are required to further compare these agents with existing oral therapies and insulin, and include hard clinical outcomes, such as cardiovascular events and mortality. Such studies will not only assess their long-term safety, but hopefully, will also elucidate the true benefit incretin-based therapies may provide, defining their role in the treatment of diabetes and the prevention of cardiovascular disease.

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