Glucagon-like peptide 1 based therapy for type 2 diabetes

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Background: **Incidence of type 2 diabetes mellitus (T2DM) has increased in young people in recent years and new therapies are required for its effective treatment. Glucagon-like peptide 1 (GLP-1) is a potent blood glucose-lowering hormone produced in the L cells of the intestine. It may be potentially effective in the treatment of hyperglycemia in patients with T2DM.**

Data sources: **PubMed database were searched with the terms "GLP-1", "incretins" and "diabetes".**

Results: **GLP-1 is a product of the glucagon gene, and its secretion is controlled by both neural and endocrine signals. GLP-1 lowers plasma glucose by stimulating insulin and suppressing secretion of glucagons, thus inhibiting gastric emptying and reducing appetite. GLP-1 exerts these actions by the engagement of structurally distinct G-protein-coupled receptors (GPCRs). In patients with T2DM, GLP-1 increases insulin secretion and normalizes both fasting and postprandial blood glucose when given as a continuous intravenous infusion. However, the native hormone is unsuitable as a drug because it is broken down rapidly by dipeptidyl peptidase IV (DPP-4) and cleared by the kidneys. Fortunately, many GLP-1 agonists or analogues and DPP-4 inhibitors have been found or developed, such as exendin-4, exenatide, liraglutide, CJC1131, vidaliptin and P32/98. Clinical trials have shown their therapeutic functions in T2DM with little adverse reaction.**

Conclusion: **A GLP-1 based therapy will be safe and effective for the treatment of T2DM.**

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Key words: **glucagon-like peptide 1;**
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Glucagon-like peptide 1 (GLP-1) is an important incretin isolated in 1985. The term incretin first used by La Barre in 1932 refers to gut-derived hormones that stimulate insulin secretion with nutrient ingestion.[1,2] Another incretin in human beings is glucose-dependent insulinotropic polypeptide (GIP).[3] Both incretins potentiate glucose-dependent insulin secretion and enhance β-cell mass through regulation of β-cell proliferation, neogenesis and apoptosis. Moreover, GLP-1 inhibits gastric emptying, glucagon secretion, and food intake.[4] Patients with type 2 diabetes mellitus (T2DM) exhibit relative resistance to the action of GIP but not glucagon-like peptide 1 receptor (GLP-1R) agonist.[4] In this review, we focus on GLP-1 physiologic and therapeutic actions in T2DM.

Physiology and pathology of GLP-1

Structure of GLP-1

The human proglucagon gene located on the long arm of chromosome 2 consists of six exons and five introns.[4] With tissue-specific transcriptional and translational processing of the proglucagon gene, the L cells of the gut synthesize GLP-1(1-37).[5,6] GLP-1(1-37) is inactive until NH_2 terminal truncation of amino acids 1-6.[7] Active peptide hormone includes GLP-1(7-36) and GLP-1(7-37) amide, of which the former is major.[4] The L cells are distributed throughout the intestine but found in greatest numbers in the jejunum, ileum colon, etc.[8]

Secretion and regulation of GLP-1

The early secretion phase of GLP-1 initiates within minutes after nutrient ingestion and may last for 30-60 minutes. This phase is likely to be regulated through a "proximal-to-distal" combination of neural and hormonal mediators.[4] The "proximal-to-distal" cycle has been proposed, but it has not been established in humans.[9] The second phase lasts for 1-3 hours possibly because of direct interaction of digested luminal nutrients with L cells.[2,7] The plasma level of bioactive GLP-1 is 5-10 pmol/L in normal fasting humans.[2]

Regulation mechanisms of GLP-1 secretion comprise nutrient, neuron and endocrine.[3] GLP-1 release results from nutrient intake.[6] For instance, the

circulating level of GLP-1 increases two- to three-fold in response to glucose intake.[2] Fats and carbohydrates can stimulate GLP-1 secretion by direct contact with the mucosa of the small intestine. In humans, a protein meal does not increase GLP-1 secretion, whereas ingestion of an amino acid mixture does.[2] The secretion of GLP-1 is also related to gastric emptying, especially the rate of nutrient ingestion into the intestine; therefore, a liquid meal results in significantly more GLP-1 release than a solid meal of identical composition.[3] Several studies have provided evidence for a role of the vagus nerve in mediating nutrient signals of the duodenum to control GLP-1 secretion of distal intestine,[2] and vagal cholinergic muscarinic regulation has been proposed.[3] The sympathetic nervous system and non-adrenergic non-cholinergic innervation have also been proposed to be involved in the regulation of GLP-1 secretion.[3] An enteroendocrine loop between the duodenum and the jejunum may be involved in regulating GLP-1 secretion.[8] Endocrine signals from the proximal intestine may also play a role in GLP-1 secretion, particularly GIP.[2]

Metabolism of GLP-1

GLP-1 secreting from gut L cells and releasing into the circulation is rapidly cleavaged by DPP-4 to GLP-1(9-36) and GLP-1(9-37) amide inducing its inactivation.[5] The half-life of intact GLP-1 is <2 minutes in humans.[7] DPP-4 is a 110 kDa plasma membrane glycoprotein ectopeptidase and ubiquitously expressed on the surface of endothelial and epithelial cells, and its highest levels in humans have been reported to occur in the intestine, bone marrow and kidney.[10] This enzyme cleaves at the penultimate alanine residue to produce an NH_2-terminally truncated product incapable of stimulating insulin release through the GLP-1 receptor.[7] Neutral endopeptidase 24.11 (NEP24.11, also known as neprilysin) is a widespread membrane-bound zinc metallopeptidase.[11] It cleaves peptides at the C-terminal region of the GLP-1 molecule and clears its metabolism.[3,10] The primary route for GLP-1 clearance appears to be the kidney.[3,4]

Physiology of GLP-1

GLP-1 augments glucose-stimulated insulin secretion, which plays an important role in the maintenance of systemic glucose homeostasis.[6] GLP-1 also enhances insulin biosynthesis via induction of insulin gene expression.[7] As GLP-1R is expressed in pancreatic α-cell, β-cell and δ-cell,[4] the inhibition of glucagons release by GLP-1 may be a direct effect or an indirect one via somatostatin release.[2,7] The inhibiting function works in a glucose-dependent manner,[12] that is, GLP-1 does not suppress glucagon secretion induced by hypoglycemia.[5] Moreover, GLP-1 positively impacts glucose homeostasis by directly regulating hepatic glucose uptake and production, and increasing glucogen synthesis, glucose oxidation and utilization.[2]

GLP-1 increases pancreatic β-cell mass by stimulating β-cell proliferation and neogenesis[13] as well as inhibiting apoptosis[13-15] and improving viability[2] in part by down-regulation of caspase-3 and up-regulation of the anti-apoptotic protein Bcl-2.[4] As an overeating factor, GLP-1 inhibits appetite and delays gastric emptying. This function may be related to the vagal nervous system.[16] Taken together, all of these effects render GLP-1 to be a potential candidate for diabetes therapy.

Mechanism of GLP-1 action

GLP-1 works by the engagement of distinct G protein coupled receptors (GPCRs).[7] Binding of GLP-1 to its receptor in β-cells results in an increase in intracellular cAMP, leading to the stimulation of insulin exocytosis by two different pathways: PKA-dependent and PKA-independent (EPAC) pathways.[17] After activation, PKA and cAMP-guanine nucleotide exchange factor II (cAMP-GEF II) is likely to mediate the number of molecular processes involved in the regulation of insulin secretion by GLP-1.[6] GLP-1 influences the β-cell membrane potential by inhibiting the K_{ATP}- and Kv-channels, thus facilitating membrane depolarization. These changes translate into activation of the voltage-gated Ca^{2+}-channels with resulting Ca^{2+} influx and initiation of Ca^{2+}-dependent insulin exocytosis.[2,6] In addition, GLP-1 inhibits the activity of Kv-channels responsible for β-cell repolarization.[2]

The anti-apoptotic effects of GLP-1 are the results of the activation of cAMP and phosphatidylinositol 3-kinase (PI3K). These two pathways are complementary. The cAMP pathway is mediated by the activation of response element binding protein (CREB) and its interaction with the coactivator TORC2 (transducer of regulated CREB activity), thereby enhancing insulin receptor substrate-2 gene expression and leading to protein kinase B (PKB) activation.[17] GLP-1 stimulates insulin gene expression by activation of nuclear factor of the activated T-cells (NFAT) and activation of extracellular signal-regulated kinase (ERK) by a mechanism dependent on mitogen-activated protein kinase kinase (MAPKK or MEK).[7,13] GLP-1 also increases the activity of pancreatic duodenal homeobox 1 (PDX1), leading to the regulation of gene expression.[17]

GLP-1 receptor activation leads to the stimulation of PI3K by the two pathways. PI3K subsequently activates its downstream targets: mitogen-activated

protein kinase (MAPK), ERK, PKCζ and PKB/Akt. In β-cells, PKC and MAPK activation is associated with GLP-1-induced proliferation, whereas ERK and MAPK activation leads to β-cell differentiation.[17] Molecular mechanisms for GLP-1 regulation of pancreatic mass are not completely understood and further studies are needed.[15]

Role of GLP-1 in the pathogenesis of T2DM

T2DM is a multifactorial disease that is characterized by hyperglycemia, insulin resistance, absolute or relative insulin deficiency, increased hepatic glucose production, and frequently accelerated gastric emptying and obesity.[18] Increased incidence of T2DM in youth has been recognized in recent 20 years.

Progressive deterioration of β-cell function is a characteristic of T2DM. From insulin resistance to overt diabetes, progressive β-cell failure can be divided into five stages. In stage 1, insulin secretion is increased to maintain normoglycemia with insulin resistance. In stage 2, as a result of reduction in the β-cell mass and disruption of function, glucose levels start to rise. Stage 3 is a transient unstable period of early decompensation, and stage 4 is characterized by a significant reduction in β-cell mass, eventually leading to the severe decompensation of stage 5. The factors contributing to β-cell failure are still not well understood.[2] Animal experiments have shown that GLPR$^{-/-}$ mice exhibit impaired glucose homeostasis. Glucose-stimulated insulin secretion is significantly decreased following oral glucose in both GLP-1R and GIPR gene knockout mice and the glucose lowering actions of DPP-4 inhibitors are extinguished.[4] Hence, incretin receptor signaling exerts physiologically relevant actions critical for glucose homeostasis[4]. A GLP-1R polymorphism in which threonine 149 is substituted with a methionine residue was recently identified in a patient with T2DM, but not in non-diabetic control subjects. Compared to the wild type receptor, the variant GLP-1R showed a 60-fold reduction binding affinities and markedly decreased potencies of the peptide in triggering cAMP-mediated signaling.[19,20] The patient with this mutation exhibited impairment of both insulin secretion and insulin sensitivity.[19] The meal-related GLP-1 response in T2DM was decreased, which may contribute to the decreased incretin effect in T2DM.[2,18] Contrary to the reports from these subjects with long-standing T2DM, late-phase GLP-1 release was augmented in newly diagnosed subjects, concomitant with rising plasma insulin levels.[9] Other studies have shown that human subjects with T2DM exhibit relative resistance to the actions of GIP, but not GLP-1R agonists,[4] suggesting GLP-1 potential therapeutic effect in T2DM.

GLP-1R agonists or GLP-1 analogues in the treatment of T2DM

Human GLP-1

GLP-1 has been studied in human diabetes since 1992 when it was shown that acute intravenous administration of the peptide to patients with T2DM reduced postprandial glucose excursion and markedly decreased meal-related insulin requirements.[2] Short- and long-term follow-up of intravenous or subcutaneous infusion of GLP-1 also showed promising reductions in blood glucose in diabetes. A 6-week course of continuous GLP-1 administration by a subcutaneous pump device in patients with T2DM not only decreased fasting blood glucose by 4.3 mmol/L and reduced hemoglobin A1c (HbA1c) by 1.3%, but also markedly increased the maximal secretory capacity of insulin.[21] *In vivo* GLP-1 treatment has been hampered by the low plasma half-life and fast renal clearance of the natural hormone.[13] Therefore, much effort has been made to the development of long-acting GLP-1 analogues that are insensitive to DPP-4 or GLP-1 agonists, known as DPP-4 inhibitors in the treatment of T2DM.

GLP-1 receptor agonists

Exendin-4 (Ex-4)

Ex-4 is a 39-amino acid peptide isolated from the salivary secretions of the Gila Monster in 1992.[15] It is a potent agonist of the GLP-1R with an *in vivo* potency reported up to 5-10 times greater than GLP-1 itself. Ex-4 shares 53% amino acid identity with GLP-1, yet it is resistant to DPP-4 cleavage.[7] This resistance is conferred by the presence of glycine at position 2.[2] Its half-life in human is 2-4 hours, allowing for twice or three times daily dosing to achieve therapeutic serum concentrations.[2] HbA1c levels are reduced in T2DM treatment with sulfonylureas and/or metformin as well as during Ex-4 monotherapy.[22,23] In addition, Ex-4 expands or maintains β-cell mass. Ex-4 treatment during the pre-diabetic state ameliorates or prevents the development of diabetes.[2] Animal experiments have proved that Ex-4 treatment delays the onset of diabetes in db/db mice.[2]

Exenatide (AC2993[24])

Exenatide (synthetic exendin-4), marketed under the commercial name of Byetta, is the first GLP-1 receptor agonist to be licensed in the USA in April 2005.[17] In both short-term and 30-week clinical studies, exenatide lowered fasting and postprandial glucose in subjects with T2DM.[2,7] Furthermore, exenatide administration does not impair the counterregulatory response at hypoglycemia.[2] Other clinical studies found that

subcutaneous injection site did not affect exenatide bioavailability.[25]

Exenatide subcutaneously injected twice daily (before breakfast and dinner) in combination with meltformin, sulfonylureas, or both of them significantly reduced the levels of HbA1c and fasting glucose in association with modest degrees of weight loss.[26-28] The most common treatment-emergency adverse event was mild to moderate gastrointestinal symptoms such as nausea, only found in 3% of the patients. Nausea occurred most commonly in the initial weeks after starting therapy but less frequently in the study progressed.[28] More severe side effects have been avoided successfully by starting with a low dose and increasing the dose at one-week intervals.[22] Exenatide treatment was not associated with an increased incidence of cardiovascular, hepatic, or renal adverse events and severe hypoglycemia.[28] 19%-22% of the patients who received exenatide developed anti-exenatide antibodies but these antibodies appeared not to influence glycemic control.[2,24]

Preliminary experience with exenatide long-acting release (LAR) with weekly subcutaneous administrations in T2DM patients indicates a much greater reduction in fasting glucose concentrations and HbA1c compared with exenatide twice daily. However, long-term experience with the drug in larger numbers of patients has not yet been reported. Exenatide LAR is currently being assessed in a phase III head-to-head trial against twice-daily exenatide.[29]

GLP-1 analogues

Liraglutide

Liraglutide is a long-acting GLP analogue that differs from native GLP-1 by a single amino acid substitution and linkage to a fatty acid side chain. This side chain allows non-covalent binding to endogenous albumin, which protects the peptide from DPP-4 cleavage.[2] Thus the half-life is extended to 11-15 hours in the human beings, a pharmacokinetic profile suitable for once-daily dosing.[2,13,22]

Clinical studies demonstrated that liraglutide improved β-cell responsiveness to hyperglycemia, increased insulin secretion and decreased plasma glucagons.[5] It also reduced fasting glucose levels, decreased postprandial glucose excursion and HbA1c levels in T2DM.[2] The common adverse effects included nausea, vomiting and headache, etc.[2,22] There was no antibody formation in the liraglutide treated patients.[30] Liraglutide and native GLP-1 inhibit islet β-cell apoptosis with the former superior to the latter.[13] Therefore, liraglutide may be useful for retaining β-cell mass in both type 1 and type 2 diabetic patients.

CJC1131

CJC1131 is another synthetic GLP-1 analogue produced by a single amino acid substitution at position 2 and by attachment of a chemical reactant at the carboxy-terminal end that allows for covalent binding to endogenous serum albumin and protects against DPP-4 degradation. Its half-life is similar to that of circulation albumin, approximately 10 to 15 days.[2,22,31] CJC1131 stimulated insulin secretion and biosynthesis, inhibited food intake, and stimulated islet neogenesis in a mouse model of T2DM.[31] Clinically, CJC1131 reduced levels of fasting and postprandial glucose and weight loss in subjects with diabetes.[2] Although recombinant albumin GLP-1 proteins such as CJC1131 and exenatide-LAR are expected to have an extended pharmacokinetic profile suitable for once weekly dosing in diabetic patients, little clinical information is available about the efficacy and safety of these albumin-based drugs in the human beings.[29]

DPP-4 inhibitors

There are now several inhibitors of DPP-4 that are presently undergoing clinical trials, including isoleucine thiazolidide (P32/98), P93/01, NVP-DPP728, vildagliptin (LAF237), 815541A, sitagliptin (MK-0431), GSK23A, Valine-pyrrolidide, and saxagliptin (BMS-477118).[2,10,32,33] DPP-4 inhibitors increase circulating GLP-1 levels, improve β-cell responsiveness to glucose, enhance insulin sensitivity in T2DM, and inhibit glucagons secretion,[10] thus reducing the levels of fasting and postprandial glucose and HbA1c.[2] Clinical studies have shown that DPP728 is a feasible approach to the treatment of T2DM in the early stage of the disease[34] and poorer metabolic control patients with higher baseline HbA1c levels of between 8% and 9.5%.[33] Ristic et al[35] confirmed that vildagliptin at a dosage of 50 or 100 mg once daily for 12 weeks can significantly reduce HbA1c levels in patients with T2DM and appears to be safe and well tolerated. DPP-4 inhibitors in combination with GLP-1 augment insulin secretion of GLP-1.[36] Other clinical studies showed that sitagliptin dose-dependently inhibited plasma DPP-4 activity, enhanced active GLP-1 and GIP levels, and decreased glucagon with no hypoglycemic events.[37] Sitagliptin and vildagliptin were registered by US FDA in 2006.[17]

DPP-4 inhibitors do not affect weight and appetite of diabetes patients compared with GLP-1 analogues, which is related to the relatively lower levels of GLP-1 achievable by DPP-4 inhibitors. In addition, DPP-4 inhibitors do not lead to nausea and can be administered orally.[38] Up to 50% of GLP-1 entering the circulation may be degraded by NEP-24.11;

therefore combined inhibition of DPP-4 and NEP-24.11 is superior to DPP-4 inhibition alone in preserving intact GLP-1, which raises the possibility that the combination has a potential therapeutic effect.[11] Oral metformin effectively inhibits DPP-4 activity in T2DM, thus reducing GLP-1 degradation.[32] Animal experiment indicates that metformin decreases the plasma DPP-4 activity, while limiting the inactivation of exogenously administered GLP-1 and improving glycaemic control.[39] The drug may be potentially used for future combination therapy with incretin hormones.[32]

However, as DPP-4 (also known as CD26) is involved in the degradation of a wild array of peptide substrates, potential side effects may accompany systemic inhibition of this ubiquitous enzyme.[2] Although clinical studies have demonstrated good tolerability without serious side effects,[2] final judgment must depend on additional long-term clinical studies.[33]

Gene therapy

A single administration of rAd-GLP-1 via the tail vein into streptozotocin (STZ)-induced diabetic non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice resulted in remission of diabetes within 10 days, and normoglycemia remained until the experiment was terminated.[40] Intramuscular gene transfer of the plasmid in db/db mice demonstrated that expression of the GLP-1/Fc peptide normalized glucose tolerance by enhancing insulin secretion and suppressing glucagon release.[41] Hence gene therapy may be a potential direction in future.

GLP-1 lowers plasma glucose in T2DM by inducing insulin secretion, increasing β-cell mass and reducing food intake. Therapy based on GLP-1 in patients with T2DM has little hypoglycemia adverse effect compared with some of other drug therapies. There are many new GLP-1R agonists, GLP analogues and DPP-4 inhibitors were not mentioned in this review, such as LY307161SR. Most clinical studies so far conducted are less than a period of one year. Demonstration of GLP-1 preservation or regeneration of β-cells in humans will need long-term, randomized controlled studies.[12] In addition, there are little clinical studies in children.

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