

Advances in GLP-1 receptor agonists for the treatment of type 2 diabetes

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Abstract. Glucagon-like peptide-1 (GLP-1), an incretin secreted by intestinal L cells, has become a critical target for the treatment of type 2 diabetes because of its physiological effects of augmenting insulin secretion, suppressing glucagon secretion, and decelerating gastric emptying. Human endogenous GLP-1 is found to be proteolytically degraded and inactivated by DPP-4, which considerably limits the therapeutic effects of GLP-1. In contrast, GLP-1RAs undergo significant improvement in drug stability. In this context, several successful strategies for the development of GLP-1RAs and the corresponding problems are fully elaborated. The assay gives a brief overview of the pharmacological effects, advantages and common adverse effects of GLP-1RAs, shedding light on the latest research progress of GLP-1RAs, including new dosage forms, new drug targets and new clinical applications.

1. GLP-1 and GLP-1RA

1.1 GLP-1

In 1985, Schmidt et al. first isolated and extracted glucagon-like peptide-1 (GLP-1) from the intestinal mucosa. GLP-1 is an incretin secreted by the terminal jejunal, ileal, and colonic epithelial L cells, as a proglucagon cleavage product by preproprotein convertase 1 (PC1) in intestinal L cells. GLP-1 recognizes and activates the GLP-1 receptor (GLP-1R) in the human body and has a wide range of physiological actions. The most obvious effect of GLP-1 is its antihyperglycaemic property which is mediated predominantly by its insulinotropic and glucagonostatic actions. In addition, the effect mentioned above is also concerned with GLP-1-induced delay in gastric emptying, stimulation of β cell regeneration in the pancreas, central nervous system induction of satiety and fullness, as well as increasing uptake of glucose by cardiomyocytes, adipocytes, and skeletal muscle. GLP-1 shows cardiovascular benefits by increasing myocardial contractility, accelerating heart rate, and improving ischemic Myocardial injury. Furthermore, the additional effects of GLP-1 include: promoting hepatic glycogen synthesis and inhibiting glycogenolysis when acts on the liver, increasing urinary sodium excretion when on the kidney. These effects make it possible to apply GLP-1 in the treatment of T2DM.

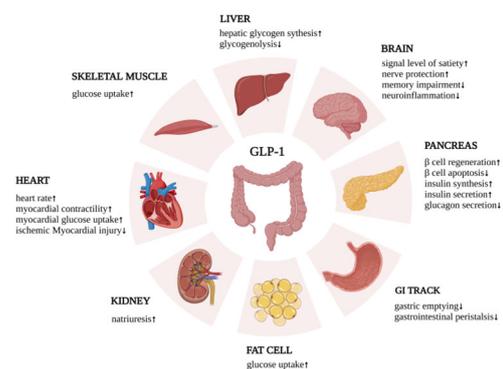


Figure 1. Physiological effects of GLP-1 on various organs and tissues

In humans, the biologically active forms of GLP-1(1-37) are mainly the following two: GLP-1(7-36) and GLP-1(7-37), the latter being the glycine-extended 31-peptide form. Of these, about 80% of the active GLP-1 is present in the form of GLP-1(7-36). However, active GLP-1(7-36) is recognized by dipeptidyl peptidase-4 (DPP-4) in the human body, undergoes a peptide chain break between Ala8 and Glu9, and is rapidly degraded to dipeptide (His-Ala) and inactive GLP-1(9-36), which is cleared from the body's circulation via the kidneys. Therefore, the half-life of endogenous GLP-1 in humans is very short, usually only 2-3 min, which greatly limits the effect of GLP-1 in the treatment of T2DM.

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peptidase-4 (DPP-4), undergoes a peptide chain break between Ala8 and Glu9, which is followed by rapid degradation to dipeptide (His-Ala) and inactive GLP-1 (9-36). Therefore, the half-life of endogenous GLP-1 in humans is very short, generally only 2-3 min, which significantly limits the application of GLP-1 in the treatment of T2DM.

In an attempt to modify GLP-1, the structure-activity relationship of GLP-1 was explored. Suzuki S et al. [89] examined the activity of the N-terminal and C-terminal fragments of GLP-1 with a rat pancreatic perfusion system, and found that GLP-1 had significant proinsulin secretory activity, whereas GLP-1 (1-37), GLP-1 (6-37), and GLP-1 (8-37) did not. For the C-terminal fraction, GLP-1 (7-37) and GLP-1 (7-36) were found to have similar proinsulin secretory activity as GLP-1 did, significantly suppressing glucagon release at specific concentrations, whereas GLP-1 (7-35) and GLP-1 (7-34) had a reduced affinity for the receptor and a lower biological activity as a result. It can be therefore assumed that His7, is a more important component that exerts the insulinotropic and glucagonostatic actions of GLP-1 compared to the three amino acids at the C-terminus (Gly37, Gly35, and Arg36). According to the GLP-1 receptor activation model theory, the C-terminal of GLP-1 initially binds to the extracellular N-terminal structural domain (NTD) of the receptor to induce a conformational change in GLP-1, thus ensuring the N-terminal of GLP-1 binding to the core region of the receptor (TMD), which is essential for the activation of GLP-1 R. [89][91] Therefore, His7 could be a major factor, if not the only one, causing the activation of GLP-1 R. Further studies corroborate this assumption, indicating that His7, Gly10, Phe12, Thr13, and Asp15 are directly involved in receptor interactions in GLP-1, while Phe28 and Ile29 play a significant role in the maintenance of the secondary structure of GLP-1 and the conformational changes of the peptide to adapt to receptor recognition. [92] These findings serve as an important basis for GLP-1 structural modification.

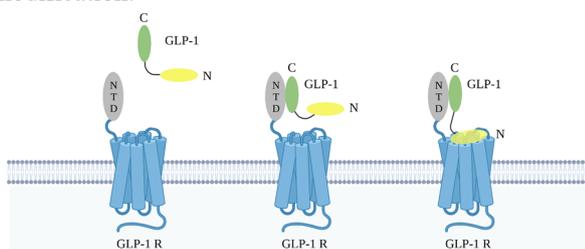


Figure 2. Schematic diagram of GLP-1 receptor activation model

1.2 GLP-1RA

In order to prolong the half-life of GLP-1 in humans, the chemical structure of GLP-1 was modified and GLP-1 analogues—GLP-1 receptor agonists (GLP-1RAs), were developed. GLP-1RAs are divided into short-acting and long-acting forms, the former include exenatide (twice daily), lixisenatide (once daily), and oral semaglutide (once daily), while the latter includes liraglutide (once daily), exenatide extended-release (once weekly),

albiglutide (once weekly), dulaglutide (once weekly) and semaglutide (once weekly). All drugs are administered by subcutaneous injection, with the exception of oral semaglutide, which is intended for oral administration. The current development strategies for GLP-1RAs include: chemical structure modification to extend the half-life of the drug; development of non-immunogenic modified materials to address injection site reactions; the development of oral delivery formulations to improve patient compliance, reduce injection site reactions and lower production costs; the development of inhalation formulations to improve patient compliance and avoid gastrointestinal irritation or degradation; the development of long-acting dosage forms and continuous drug injection systems to reduce the frequency of administration, etc. Of these, the major strategies for prolonging the half-life of GLP-1RAs are as follows.

1.2.1 Replacement of Ala8 to block the cleavage site of DPP-4

Exenatide is structurally identical to Exendin-4, a peptide hormone isolated from the venom of the Gila monster. Although exenatide has merely 53% amino acid sequence identity with GLP-1, in vitro study has shown that it is even slightly more potent than GLP-1, which could be the case therefore that both of them share high similarity in the N-terminal key amino acid residues. As a major improvement over endogenous GLP-1, exenatide substitutes Ala8 for Gly8 in GLP-1, and resists degradation of DPP-4. Consequently, the half-life of exenatide is extended to 2.4 hours and is approved for subcutaneous administration twice daily. Similarly, lixisenatide also changes Ala8 to Gly8, and adds six Lysines after Ser45 at the C-terminal to withstand DPP-4-mediated degradation, further extending the half-life to 2.6h. [90] Both lixisenatide and exenatide are short-acting drugs, but the former has a higher capacity to delay gastric emptying, [90] reducing the frequency of administration to once daily. However, it has been found that the introduction of Gly8 improves the stability of GLP-1 to DPP-4 while significantly reducing its affinity for GLP-1 R. Subsequent drug development attempts found that the only Ala substitution that achieved a balance between DPP-IV stability and high GLP-1R affinity was the introduction of aminoisobutyric acid (Aib) at position 8. [135]

1.2.2 Introduction of fatty acid chains for preparation of peptide-albumin complexes

Human serum albumin (HSA) is a major protein in human blood, which is involved in drug transport in vivo. HSA has good compatibility and wide distribution within the human body, without immunogenicity or enzymatic activity. High-affinity binding of HSA to neonatal receptor (FcRn) recycles the protein back to plasma, extending the half-time of the drugs which are combined with fatty acid and linker to maximize HSA binding. Accordingly, human serum albumin fusion technology has become a common technique for the development of

long-acting GLP-1RAs, mainly including non-covalent coupling, chemical covalent coupling and fusion protein technology.

In addition to the substitution of Arg34 for Lys34, liraglutide uses non-covalent coupling by introducing a Glu-mediated palmitic acid at position 26 to form a peptide-albumin complex through hydrophobic and inter-ionic interactions between palmitic acid and albumin. The peptide-albumin complex can produce a large spatial barrier to the enzymatic cleavage of DPP-4, with a larger molecular weight of the peptide-albumin complex slowing down the renal clearance rate, the half-life of the drug is extended to 11-13h. [4] Likewise, Semaglutide also introduces an 18-carbon fatty acid side chain at position 26 and changes Ala8 and Lys34 to Aib8 and Arg34, respectively, with a half-life of up to 47h. [4]

However, it has been suggested that albumin competes with GLP-1R on GLP-1RA binding, and the stronger the long fatty acid's binding capacity to HSA, the less free fraction of GLP-1RA, which means a lower drug efficacy, and an increased dose is required to achieve the desired efficacy.¹¹⁹ This is evidenced by the higher injectable dose of liraglutide compared to other GLP-1RAs, where the starting dose of liraglutide is 0.6 mg once daily and needs to be increased to 1.2 mg per dose after about a week, with a maximum clinical dose of 1.8 mg. The starting dose of exenatide is 5ug twice daily and can be increased to 10ug after 1 month depending on the clinical responses. Consequently, achieving a balance between a longer plasma half-life and a higher GLP-1R affinity remains a difficult issue at this time.

1.2.3 Albumin fusion

Albiglutide is a recombinant protein composed of two GLP-1 analogues and human albumin, and both GLP-1 analogues undergo Ala8 to Gly8 substitution, which enhances the resistance of the drug to DPP-4 degradation. As mentioned above in 1.2.1, albumin fusion can further extend the half-life of albiglutide to 6-8 days through increased steric interference with DPP-4 degradation and increased molecular weight to reduce renal filtration. Albiglutide is approved for subcutaneous dosing on a weekly basis. The method of albumin fusion is part of the human serum albumin fusion technology as well as non-covalent coupling, which extends the half-life of the drug while creating a larger steric hindrance in albumin that may also affect the binding of the drug to the receptor. [111] In addition, the large molecular weight of the fusion protein restricts the drug mainly to the circulatory system, affecting its ability to penetrate peripheral organs and tissues, which results in a reduced efficacy of albiglutide. [121]

1.2.4 Fusion Fc fragment

Dulaglutide is a homodimeric fusion peptide, each monomer consisting of two GLP-1 analogs and a hIgG4-Fc fusion. As with albiglutide, the GLP-1 analogue undergoes an Ala8-to-Gly8 substitution, improving the degradation resistance of DPP-4. The difference is that the

GLP-1 analogue is linked to the N-terminal end of the Fc in hIgG4-Fc with a 3-segment repeat of the GGGGS (G4S) amino acid sequence. [92] As a long half-life (2-4 weeks in humans) protein, IgG can bind to the neonatal receptor (FcRn) in acidified endosomes and escape lysosomal degradation. It can be released into the extracellular circulation at physiological pH to return to the circulation, thus prolonging the circulation of the fusion peptide *in vivo*.⁴ In addition to the FcRn-mediated recirculation mechanism, Fc fusion increases the molecular weight of the peptide to reduce renal filtration. The large volume of IgG4 fragments also creates a steric hindrance to DPP-4 degradation,⁶ all of which are beneficial for prolonging the half-life of the drug.

As an Fc fusion protein analogue for the treatment of T2DM, special attention should be paid to the Fc Y R binding domain-induced cytotoxic (ADCC) and complement activation (CDC) effects of the Fc segment. Therefore, the researchers made chemical modifications on specific amino acid sites in dulaglutide as well as mutated specific positions of the Fc structural domain, including replacing Arg36 of GLP-1(7-37) and Phe234, Leu235, and Ser228 of the heavy chain of the hIgG4-Fc fragment with Gly36, Ala234, Ala235, and Pro228, respectively, and removing Lys from the C-terminal of hIgG4-Fc, which reduced the binding of hIgG4-Fc to its receptor and improved safety of the drug.⁹³

1.2.5 Slow-release drug delivery systems

Exenatide extended-release encapsulates the drug in injectable microspheres of polylactic acid-hydroxyacetic acid (PLGA) with a diameter of 0.06 mm. After injections into subcutaneous adipose tissue, exenatide is slowly and continuously released, with PLGA polymer experiencing ester bond hydrolysis and gradual degradation into lactic acid and glycolic acids, which are eliminated in the form of carbon dioxide and water. This slow-release delivery system reduces the number of injections required to maintain blood levels and is approved for once-weekly dosing, significantly improving patient compliance. It is also possible to monitor the rate of drug release. For example, a smaller drug particle size can reduce the initial release rate; excipients can affect the porosity of the microspheres and/or the rate of polymer breakdown, either by changing the local pH conditions or catalyzing ester hydrolysis. Modification of the PLGA matrix may also have an impact on drug delivery. By increasing the propylene-to-glycine ratio in the matrix, the general hydrophilicity of the microspheres can be decreased, reducing the biodegradation rate. In contrast, the addition of carboxylic end groups increases hydrophilicity and speeds up the release of the drug.⁷

Due to foreign body reactions, lumps can be observed at the injection site after subcutaneous injection of microspheres, which is one of the most common adverse effects of long-acting exenatide.⁸ In addition, it was found that PLGA particles could not deliver the drug at a stable rate.⁹ To address this issue, researchers further developed other devices such as the implantable ITCA 650 micro-osmotic pump, an extended-release and controlled-release

formulation, in which exenatide is released in zero steps, enabling stable and continuous drug delivery. In contrast to exenatide extended-release agent, which takes about 10 weeks to achieve stable blood levels, the ITCA 650 achieves this goal within 5 days of implantation and remains stable for the duration of treatment.⁹

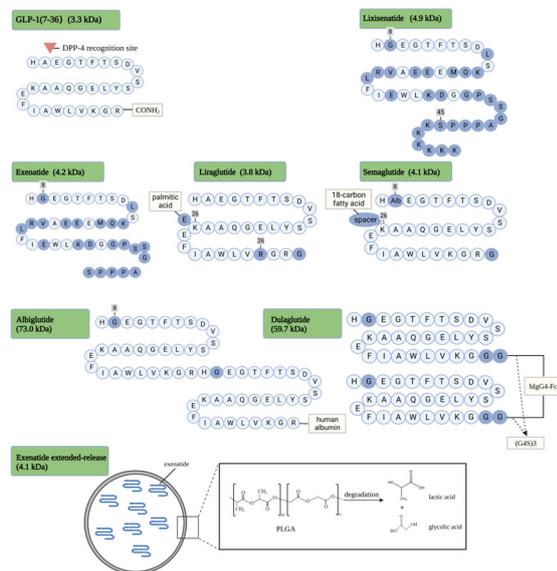


Figure 3. Schematic diagram of GLP-1RAs structure

Currently, long-acting GLP-1RAs research focuses mainly on two areas: amino acid modification and complex construction. The combined use of multiple methods to achieve optimal receptor affinity, drug stability, and safety has become a fundamental requirement for structural modification, which is also a key concern in the current research field. In addition to the five successfully applied methods mentioned above, the formation of cyclic peptides and the replacement of natural L-type amino acids with D-type amino acids not easily recognized by proteases may be expected to improve the stability of peptides and prolong the half-life of drugs.

Cyclic peptides are rich in biological activities, and the proven pharmacological effects include antibacterial, anticancer, antiviral, immunosuppression, trypsin-like activity inhibition, etc. [122] Compared with linear peptides, cyclic peptides lack amino and carboxyl-terminal functional groups, being more resistant to DPP-4 mediated degradation, which can be used for modification of long-acting GLP-1RAs based on this structural property. Considering that the cyclic peptide formed by linking the head and tail of the peptide has a great steric hindrance, which affects the binding to the receptor, the disulfide bonding method can be preferentially considered. Two cysteine residues are introduced at the designated positions (generally n, n+4 positions) of the peptide, and the disulfide bond formed by the oxidized side chain sulfhydryl group can be used as a small ring intramolecularly, which protects the DPP-4 recognition site. Despite this, the disulfide bond is unstable under reducing conditions, and the synthesis

process suffers from low yields and isomerization. [123] Furthermore, to protect the DPP-4 recognition site from peptide bond hydrolysis between Ala8 and Glu9, it is theoretical to introduce cysteine at positions 7, 11, or 8, 12. However, His7 and Phe12 are essential amino acids of the drug for the hydrophobic interaction with the extracellular domain of GLP-1R, replacement with cysteine may reduce the affinity to the receptor. In addition, unnatural amino acids and cyclization are more challenging to synthesize, and further studies are needed to determine whether the biological activity will be affected while improving its peptide stability.

2. Pharmacological effects of GLP-1RA

Nowadays, T2DM is the third most common noncommunicable disease in the developed world, behind cardiovascular disease and cancer. The number of adult diabetic patients in China is increasing annually, and the prevalence rate has reached 11.9%, among which T2DM patients account for about 90%. [10] The main pathological characteristics of T2DM are relative insufficiency of insulin secretion, excessive secretion of glucagon, reduction of intestinal incretin secretion, reduced glucose absorption, and increased lipid catabolism. It has been found that GLP-1RAs can improve the symptoms of significantly elevated postprandial blood glucose in diabetic patients; while delaying gastric emptying, slowing down the rate of nutrients from food into the blood, increasing satiety, and reducing energy intake by affecting the central nervous system. In addition, GLP-1RAs can promote insulin secretion by pancreatic β cells and inhibit glucagon secretion by pancreatic α cells, which can be used as a routine hypoglycemic drug for T2DM patients, with good clinical value and application prospects.

2.1 T2DM and insulin

Insulin is an important hormone that regulates glucose homeostasis. The main initiation steps of glucose-mediated insulin secretion are the closure of ATP-sensitive potassium channels (KATP channels) and depolarization of β cells. When the blood glucose level is above normal, glucose molecules are transported into the cell by the glucose transporter (GLUT) and become phosphorylated, converted into pyruvate to enter the tricarboxylic acid cycle. This process directly leads to an increase in the intracellular ATP/ADP ratio. The KATP channel is in a state of closure due to the high concentration of ATP, which causes the depolarization of β cells. Subsequently, the L-type voltage-gated Ca^{2+} channel (VDCC) is activated, and a large amount of extracellular Ca^{2+} flow inward, promoting the release of insulin stored in the pancreas vesicles. [95] The reduced number of pancreatic β cells, suppressed secretory function, and insulin resistance in peripheral tissues in patients with T2DM all diminish the hypoglycemic effect of insulin. [94]

2.2 GLP-1RA promotes insulin secretion

In pancreatic β cells, the binding of GLP-1 or GLP-1RAs to GLP-1R activates adenylate cyclase (AC), converting ATP to cAMP. Protein kinase (PKA) and guanine nucleotide exchange factor (Epac), which are effectors downstream of AC, play an essential role in GLP-1-mediated insulinotropic secretion. The KATP channel sulfonylurea receptor subunit, SUR1, is phosphorylated by PKA and has a reduced affinity to ADP, thus promoting channel closure and cell depolarization.¹² The increased concentration of cAMP activates Epac, decreasing the ATP concentration required to close the KATP channel. The one for activation of VDCC and endocytosis of Ca^{2+} is also reduced, facilitating the increase in intracellular calcium ion levels and promoting the release of insulin stored in pancreatic vesicles.¹³ In addition, GLP-1RAs can promote insulin secretion by inhibiting voltage-dependent K^+ efflux, delaying membrane repolarization, and prolonging Ca^{2+} endocytosis via VDCC.⁹⁶

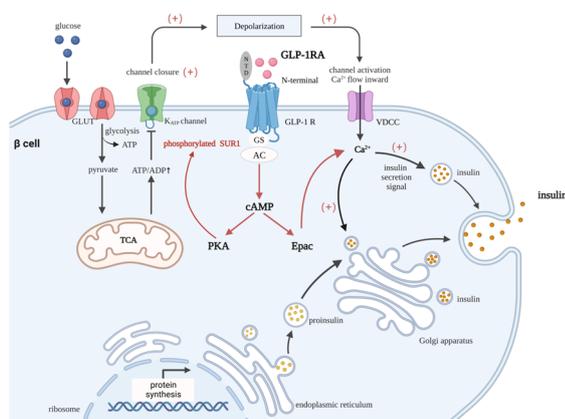


Figure 4. Mechanism of GLP-1RAs promoting insulin secretion

2.3 T2DM and glucagon

Glucagon is secreted by pancreatic α cells and acts mainly on the liver via the portal system by binding to the glucagon receptor (GCGR). Glucagon helps promote the expression of gluconeogenesis and glycogenolysis-related genes by activating the cAMP-PKA signaling pathway; Meanwhile, the expression of gluconeogenesis-related genes is also enhanced by activating the downstream phospholipase C (PLC) -inositol triphosphate (IP3) signaling pathway.

Therefore, the primary physiological role of glucagon is to raise blood glucose by promoting hepatic glycogenolysis and gluconeogenesis. [14] The long-term hyperglycemia of T2DM patients weakens the sensitivity of α cells to blood glucose, and the continuing exposure of α cells to high concentrations of insulin leads to insulin resistance, which diminishes the antagonistic effect of insulin on glucagon. As a result, more glucagon is secreted from α cells in T2DM patients, causing a high level of glucagon secretion for a long time.

2.4 GLP-1RA inhibits glucagon secretion

It is well-documented that GLP-1 can effectively suppress the secretion of glucagon.¹⁵¹⁷ This effect occurs only when the glycemia is at or below normal. When blood glucose level falls within the hypoglycemic range (≤ 3.7 mmol/L), GLP-1 does not affect glucagon secretion, which is the same with GLP-1RAs.¹⁶ However, the specific mechanisms by which GLP-1RAs inhibits glucagon secretion are relatively complex. As a potent inhibitor of glucagon secretion, growth inhibitory hormone can be secreted by δ cells with the dose-dependent stimulation of GLP-1RAs, inhibiting glucagon secretion through a paracrine mechanism.¹⁸ In addition, GLP-1RAs stimulates β cells to secret insulin, glucagon, zinc, and γ -aminobutyric acid (GABA), all of which can theoretically inhibit glucagon release.¹⁹

3. Advantages of GLP-1RAs

As T2DM is a chronic disease, patients have to take medication for a long time and often combine multiple drugs to achieve a glucose-lowering effect. Thus, polypharmacy, which can result in unpredictable drug interactions and adverse effects should be paid extra attention to. Compared with traditional hypoglycemic agents such as insulin, sulfonylureas, biguanides, and α -glucosidase inhibitors, GLP-1RAs have significantly fewer adverse effects which are restricted to certain organs or tissues, making it easier for specific treatment.

3.1 Lower risk of hypoglycemia

Hypoglycemia is one of the most frequently reported adverse events in patients with T2DM during treatment. When received glucose-lowering therapy, such as glimepiride (sulfonylurea, an oral insulin secretagogue), the powerful and long-lasting glucose-lowering effect can lead to symptoms of seizure or coma shock, which threaten the health and life of patients. In contrast, GLP-1RAs have a lower risk of hypoglycemia, which is related to the glucose-dependent proinsulin secretory mechanism of GLP-1RAs.

GLP-1RAs can promote insulin secretion when blood glucose is elevated, and do not exert this effect when patients' blood glucose levels fall below normal. A significant increase in intracellular cAMP content, adenylate cyclase activity, and insulin release was observed after Exendin-4 stimulation under 25 mmol/L glucose culture condition ($P < 0.05$). In contrast, there were no significant changes under 2.8 mmol/L glucose culture conditions ($P > 0.05$) [97]. This finding was also supported by Degen KB et al. in their study of the hypoglycemic effect of exenatide [98]. T2DM patients who received treatment with metformin were enrolled in a 26-week open-label study. Patients were randomly assigned to receive exenatide twice daily (BID) or premixed insulin aspart 70/30 BID (PIA). The results showed that exenatide (bid) was not inferior to PIA in terms of glycemic control, but was significantly better than PIA concerning the incidence of hypoglycemia and weight control.²⁰ Another clinical study demonstrated that lixisenatide can effectively control blood glucose, and

is highly effective in treating patients with elevated blood glucose due to relatively insufficient postprandial insulin secretion. Patients who received the treatment of lixisenatide could achieve weight loss within 24 weeks and had no risk of severe hypoglycemia.²¹ The results of a clinical study on Chinese T2DM patients showed that the proportion of patients who did not gain weight or did not experience hypoglycemia after achieving the treatment goal of HbA1c < 7.0% was significantly higher in patients using liraglutide compared with glimepiride (GLIM) and glargine insulin (GLAR).²² In another study of East Asian patients (77% Chinese population), the incidence of hypoglycemia was significantly lower with dulaglutide 1.5 mg (5.7%) and dulaglutide 0.75 mg treatment (3.6%) than with the administration of glimepiride (15.6%).⁹⁹ In addition to the lower risk of hypoglycemia, it was also shown that the administration of GLP-1RAs during hypoglycemia counteracted the endothelial dysfunction, oxidative stress, and inflammatory response the hypoglycemia triggered, which are likely to be related to the antioxidant activity of GLP-1RAs²³.

3.2 Significant weight loss effect

Obesity and overweight are independent risk factors for T2DM, and the degree of obesity, fat-centric distribution, and increased body mass are all risk factors for triggering T2DM. Dysfunctional obese adipose tissue can release excessive free fatty acids, reactive oxygen species, and pro-inflammatory cytokines, which further induce the development of insulin resistance.²⁴ Under the circumstances of insulin resistance, the insulin sensitivity of pancreatic β cells and glucose utilization in skeletal muscle, adipose tissue, and liver are continuously reduced, bringing about an elevated blood glucose level, and compensatory secretion of insulin. The continuous overload can deplete β cells and worsen the condition of patients.

Several studies have shown that liraglutide can forcefully reduce body weight while controlling blood glucose. In a randomized, double-blind, placebo-controlled 20-week study, participants received diet and exercise counseling before being randomly assigned to once-daily subcutaneous liraglutide, open-label orlistat, or placebo. After one year, the liraglutide recipients lost 5.8 kg more weight than those on placebo and 3.8 kg more than those on orlistat. In the second year, nearly 70%, 43%, and 25% of the participants using liraglutide maintained weight loss >5%, >10%, and >15% of screening weight, respectively, indicating that liraglutide was able to keep the weight loss effect.²⁵ In another 26-week randomized trial, T2DM patients were randomly assigned to receive a combination of glimepiride (2-4mg/day) and liraglutide, rosiglitazone, or placebo. The reduction in body weight was slightly more with the administration of liraglutide (-0.2kg) than with placebo treatment (-0.1kg), and liraglutide showed an advantageous weight loss effect when compared with rosiglitazone (+2.1kg).²⁶ This also demonstrated the weight-reducing effect of liraglutide. A note of caution is due here since the patients receiving liraglutide 0.6 mg and 1.2 mg once per day gained weight

of 0.7 kg and 0.3 kg, respectively, suggesting the weight-reducing effect of liraglutide may be related to the dosage. Davies M et al. treated overweight or obese people and T2DM patients with once-weekly (2.4 mg) subcutaneous injections of semaglutide and found that the weight-reducing effect of semaglutide was more pronounced compared with placebo.²⁷ In comparison with patients taking a placebo, patients receiving semaglutide demonstrated a 24% reduction in the total energy intake throughout the day, and more decrease in mean body weight after 12 weeks (-5.0 kg versus +1.0kg) has been reported in patients treated with semaglutide and placebo.²⁹ Furthermore, exenatide can suppress the desire for high-calorie foods, whose weight loss effect was similar to that of a low-calorie diet.²⁸ In addition, dulaglutide has the same therapeutic effect of weight loss.^{22,99}

3.3 Better treatment effect for complications

3.3.1 GLP-1RA and cardiovascular disease

Patients with diabetes have around twice the risk of cardiovascular disease as non-diabetic patients when other conventional risk factors are excluded.³⁰ About one-third of patients with T2DM have cardiovascular disease or are at risk for such sickness.³¹ Early studies found that nitric oxide-mediated vasodilation is impaired in T2DM patients.³² In patients with T2DM, the blood is prone to hypercoagulability and hypofibrinolysis.³³ In addition, patients with T2DM have a greater volume of atherosclerosis, smaller arterial lumen, higher incidence of thrombosis, and faster progression compared to non-diabetic patients,³⁴ which remarkably increases the incidence of cardiovascular disease in T2DM patients.

GLP-1RAs can increase endogenous cellular antioxidant concentrations, inhibit oxidative stress-mediated cell injury, reduce cardiomyocyte apoptosis, and improve endothelial repair and revascularization in T2DM patients by promoting endothelial cell multiplication and migration, and exerting cardiovascular benefits. [39] A meta-analysis showed that GLP-1RAs have a favorable cardiovascular safety profile, reducing major adverse cardiovascular events (MACE), cardiovascular mortality, and all-cause risk mortality.³⁷ In a double-blind trial, patients with T2DM and high cardiovascular risk were randomly assigned to receive liraglutide or placebo, with a median follow-up time of 3.8 years. The results showed that all-cause mortality (8.2% versus 9.6%) and cardiovascular mortality (4.7% versus 6.0%) were lower in patients treated with liraglutide than in those receiving placebo, and the incidence of nonfatal myocardial infarction and nonfatal stroke was both lower in patients receiving liraglutide than in those using placebo.³⁵ A trial comparing the cardiovascular safety of liraglutide and basal insulin in patients with T2DM and high cardiovascular risk demonstrated that liraglutide was associated with a significantly lower risk of MACE (4.74% to 2.91%) while using basal insulin could be related to a higher cardiovascular risk.^{36,36]}

Another research has shown that exenatide helps to manage cardiovascular risk and complications by reducing body weight, glycated hemoglobin, and blood pressure.³⁷ Bunc MC et al. randomized T2DM patients treated with metformin to receive exenatide or glargine insulin. The results showed that exenatide could reduce body fat mass and improve circulating biomarkers of cardiovascular risk after one year of treatment; while no significant changes were seen in the glargine insulin group, except for a trend toward lower endothelin-1 levels.³⁹ In addition, the SUSTAIN-6 and PIONEER-6 studies concluded that semaglutide also able to reduce cardiovascular risk. A post hoc analysis reported that semaglutide reduced cardiovascular adverse events by approximately 24% compared to placebo.⁴¹

3.3.2 GLP-1RA and chronic kidney disease (CKD)

Chronic kidney disease (CKD) is one of the most common complications of T2DM, and approximately 40% of patients with diagnosed or undiagnosed diabetes have CKD.⁴² The principal manifestation of CKD is an increase in urine albumin and/or a persistent decrease in the glomerular filtration rate. GLP-1RAs have been shown to have renal benefits and delay the progression of CKD.⁴³ The main pharmacological effect of GLP-1RAs is to reduce the occurrence of massive albuminuria [urine albumin-creatinine ratio (UACR) >300 mg/g] and slow the decline in eGFR in diabetic patients, presumably related to the lowering of blood glucose, blood pressure, and weight loss by GLP-1RAs. Compared with other hypoglycemic drugs, GLP-1RAs have a wide range of applicability, especially liraglutide, albiglutide, and dulaglutide, all of which can treat stage 2 and 3 CKD without dose adjustment, while traditional drugs such as metformin and SGLT-2 inhibitors can only achieve the same effect in the treatment of stage 2 CKD.⁴⁷

The renal benefits of the GLP-1RAs differed from each other. In the ELIXA trial, 6068 T2DM patients with a history of myocardial infarction or unstable angina were randomized to receive lixisenatide (10-20ug) or placebo of the same volume, with a median follow-up time of 108 weeks. Among the patients, 389, 1148, and 4441 had macroalbuminuria (UACR>300mg/g), microalbuminuria (UACR30-300mg/g), and normal albuminuria, respectively. Under the treatment of lixisenatide, the UACR was decreased by 39.18% (P=0.0070) in macroalbuminuric patients. A reduction of 21.10% (P=0.0502) and 1.69% (P=0.7398) was observed in microalbuminuric and normoalbuminuric patients receiving lixisenatide, respectively, showing no significant difference. The results indicated that lixisenatide could effectively slow down the progression of macroalbuminuria rather than microalbuminuria.¹⁰⁰

Another short-term study showed that liraglutide reduced urine albumin excretion and increased urine sodium excretion. [44] However, eGFR appeared to be unaffected by liraglutide, confirmed by the data from the LIRA-RENAL trial, which addressed the effects of liraglutide in T2DM patients with moderate renal impairment.[136] A posthoc analysis of the SUSTAIN 1-7 trial found that

semaglutide could consistently reduce albuminuria levels in patients with T2DM compared to placebo.¹⁰¹ In the AWARD-7 trial, T2DM patients with moderate-to-severe chronic kidney disease (stage 3-4) were randomly assigned to once-weekly dulaglutide or daily glargine insulin. Both the drugs showed similar effects in glycemic control, but the eGFR was higher in patients receiving dulaglutide after 26 weeks of treatment than in those who received glargine insulin (34.0 mL/min /1.73 m² versus 31.3 mL/min /1.73 m², respectively). The results suggested that dulaglutide had an increased capability of slowing down the decline in eGFR and could be safer for T2DM patients with moderate to severe chronic kidney disease.⁴⁶ However, the renoprotective effects of GLP-1RAs in the treatment of T2DM have been evaluated in large clinical studies with cardiovascular outcomes as the primary endpoint event. Further confirmation is necessary in future clinical studies where renal outcomes are the primary endpoint event.

3.3.3 GLP-1RA and diabetes-related foot ulcer (DFU)

Diabetic foot ulcer is among the most common complications of diabetes mellitus, and approximately 15% of diabetic patients develop foot ulcers during the disease. Common risk factors include poor glycemic control, peripheral neuropathy, peripheral vascular diseases, and immunosuppression. There are few studies on GLP-1RAs for treating DFUs, and no definitive conclusions have been drawn.^{Seo E et al.}⁴⁸ found Exenatide-4 to be effective in treating diabetic skin wounds, and topical Ex-4 combined with adipose-derived stem cells (ADSCs) injections were more effective. Therefore, the combination of Ex-4 and ADSCs may be an operative treatment option for diabetic wounds (e.g., foot ulcers). However, whether this treatment option can be used for clinical treatment has not been proven experimentally. Furthermore, it has been shown that liraglutide treatment in T2DM patients and patients at high risk of CV events does not increase the incidence of DFUs, and the risk of amputation associated with DFUs is significantly lower than in placebo treatment.⁴⁹ Thus, further studies are needed to prove whether this association is necessarily present.

4. Adverse effects of GLP-1RA

4.1 Common adverse reactions: gastrointestinal reactions, central and peripheral nerve damage, hypoglycemia, skin side effects

Gastrointestinal reactions such as nausea, vomiting, and diarrhea are the most common adverse effects of GLP-1RA.⁵⁰ However, these effects are dose-dependent and are usually more pronounced at the beginning of treatment, with a gradual decrease in associated symptoms after continued treatment; Meanwhile, adverse drug reactions occur more frequently in short-acting drugs than in long-acting drugs, presumably related to the lesser effects of long-acting GLP-1RAs on gastric motility.⁵¹ Starting

with small doses and gradually increasing them can help improve the patient's gastrointestinal tolerance to the drug and reduce the adverse effects. Central and peripheral nervous system damage is also an undesirable effect, with trials reporting adverse effects in headache, but this reaction is usually mild and does not lead to discontinuation of the drugs.⁵¹

In addition, special attention should be paid to hypoglycemia and skin side effects at the injection site when receiving the treatment of GLP-1RAs. As mentioned above in 3.1, the risk of hypoglycemia triggered by GLP-1RAs alone is relatively low. However, clinical studies have shown that GLP-1RAs combined with sulfonylureas can slightly increase the risk of mild hypoglycemia.⁵² Therefore, when T2DM patients treated with sulfonylurea start to use GLP-1RAs, they are advised to reduce the dose of sulfonylureas appropriately to reduce the risk of hypoglycemia. Furthermore, GLP-1RAs are mainly administered by injection, and rash, erythema, or pruritus at the injection site are among the most common adverse reactions as a result. It has been found that injection site reactions occur more frequently in long-acting GLP-1RAs than in short-acting drugs, and the specific frequency differs among GLP-1RAs.⁵³ However, most reactions are mild and transient and do not cause treatment termination. Despite this, it is still essential to extend the half-life of existing drugs to reduce the number of injections. Further developing non-injectable drugs or improving the physicochemical properties of the drugs to reduce such adverse reactions is also of great significance.

4.2 Other adverse effects: pancreatitis, pancreatic cancer, fracture

As GLP-1RAs developed further, more studies began to report adverse effects of the drugs in other areas, such as promoting the development of pancreatitis, pancreatic cancer, and fractures, but there were large differences between the experimental results of different studies, and therefore the above-mentioned reactions have not been identified as adverse effects of GLP-1RAs.

Some findings suggest that GLP-1RAs are potentially harmful to pancreatic tissues.⁵⁴¹⁰⁵¹⁰⁶ However, two large meta-analyses showed no increased risk of acute pancreatitis with the treatment of GLP-1RAs.¹⁰³¹⁰⁴ Overall, there still exists a consensus that GLP-1RAs have no direct causal relationship with pancreatitis.⁵⁵ In addition, some studies have linked GLP-1RAs to an increased incidence of cancers such as pancreatic cancer. A cross-sectional study from the FDA Adverse Event Reporting System database found that exenatide treatment increased the risk of pancreatic cancer by 2.9 times compared to control drugs.¹⁰⁸ However, the conclusions drawn were inaccurate because the underlying patient profile in the study was incomplete, failing to exclude the effects of obesity, smoking, alcohol consumption, chronic pancreatitis, and concomitant use of other drugs.¹⁰⁹ Another safety assessment of liraglutide, sponsored by Novo Nordisk, showed that liraglutide did not increase the risk of pancreatic cancer compared to non-GLP-1 drugs.¹¹⁰ However, the selected follow-up period of 15 months is relatively short for studying the development of

pancreatic cancer, so further studies with a lengthened period are needed. [111] Apart from this, some researchers have demonstrated the proliferation-inhibiting and apoptosis-promoting effects of liraglutide on human pancreatic cancer cells. However, more experiments are required to verify this conclusion.⁵⁸ In terms of fracture, a meta-analysis found a negative effect of exenatide on the incident bone fractures;¹⁰⁷ While a study of exenatide and fracture incidence found no significant difference in bone mineral density between the T2DM patients treated with exenatide or metformin after 44 weeks ($P = 0.782$).⁵⁶

5. Recent research advances in GLP-1RA

5.1 New dosage forms: from injection to oral, inhalation and implantable devices

5.1.1 Oral GLP-1RA analogues: oral semaglutide

The results of several studies have shown that patients prefer oral or inhaled drugs over injections. One study found that patients with diabetes were more willing to pay more for the preferred mode of administration (e.g., inhaled insulin rather than injectable insulin) when compared with general population respondents.⁵⁹ For diabetic patients, oral medications are portable and easy to take without time and space constraints, which can remove the psychological burden and adverse reactions associated with injections and improve adherence. Therefore, developing oral GLP-1RAs as a non-injectable dosage becomes a continuing concern in the research area. However, drug development has long been technically limited by the low bioavailability of oral protein and peptide drugs due to the acidic environment in the stomach and the presence of pepsin, as well as the limited ability of protein and peptide drugs to pass through the epithelium of the gastrointestinal tract and barriers such as the small intestinal epithelial barrier and efflux pumps.⁶⁰ Several successful attempts have been made by researchers to improve the bioavailability of GLP-1RAs, including structural modifications, enzyme inhibitors, absorption enhancers, and the introduction of carrier systems.⁶⁰ Among them, the use of absorption enhancer N-(8-[2-hydroxybenzoyl] amino) sodium octanoate (SNAC) co-formulated with the GLP-1 analogue semaglutide in the form of tablets for oral semaglutide became the first oral GLP-1RAs. SNAC is a small fatty acid derivative that locally raises the pH and protects semaglutide from protease hydrolysis in the stomach, while promoting the absorption of semaglutide through the gastric epithelium in a concentration-dependent manner.⁶¹ Since relevant experiments have shown that the presence of food can hinder the absorption of this drug, oral administration of semaglutide needs to be taken after an overnight fast and for at least 30 min after administration.⁶²¹²⁰

Relevant clinical studies have demonstrated that oral semaglutide is effective in controlling blood

glucose¹¹⁷¹¹⁸ and reducing body weight.¹¹⁴¹¹⁵¹¹⁶ In addition, oral semaglutide is well tolerated and safe, with the most common adverse effects being gastrointestinal reactions. In addition, semaglutide is associated with a low risk of hypoglycemia,⁶² and cardiovascular safety is no less than placebo, similar to that of subcutaneous semaglutide. Currently, oral semaglutide has been approved by the U.S. Food and Drug Administration for the treatment of adult T2DM in September 2019 and by the European Medicines Agency. On May 27, 2022, Novo Nordisk semaglutide tablets marketing application was officially accepted by the State Drug Administration, becoming the first oral formulation of GLP-1RA declared for marketing in China. In addition, ORMD-0901, which wraps exenatide with protease inhibitors and absorption enhancers in capsules for oral use has entered phase II clinical trials [64][65], but no relevant clinical data have been published subsequently.

5.1.2 Oral GLP-1RA analogs: non-peptide small molecules

To address the many limitations of oral semaglutide in terms of dosing time and diet, non-peptide oral small molecule GLP-1RAs have become another research focus for oral drugs in recent years. Up to now, PF-06882961, PF-07081532, and LY3502970 have successfully entered clinical studies, and all of them have shown comparable safety, tolerability, and glucose-lowering and weight-loss effects with GLP-1RA injections. In the phase I clinical trial of PF-06882961, T2DM patients were randomized to receive a placebo or PF-06882961 15 mg, 70 mg, and 120 mg twice daily. After 28 days of dosing, significant reductions in fasting glucose levels, glycosylated hemoglobin (HbA1c) levels, and body weight were observed. In the 120 mg dose group, fasting blood glucose decreased by up to 90 mg/dL, HbA1c decreased by 1.2%, and body weight decreased by 8 kg.¹²⁴ PF-07081532 is an iteration of PF-06882961, which is expected to be administered orally once daily. The data from the completed Phase I clinical trial have shown a significant reduction in fasting glucose and a dose-dependent effect of weight loss.

5.1.3 Inhalation of GLP-1RA-like drugs

GLP-1 receptors are abundantly expressed in lung tissue, and the large surface area of the lung bed allows for rapid uptake of GLP-1. According to the above reasons, the researchers developed the GLP-1 inhalation powder MKC253 based on Technosphere™ technology, which adsorbs the drug onto Technosphere™ particles composed of fumaryl diketopiperazine (FDKP). Inhaled powders range in size from 2 to 5 μm and have a uniform particle size, making them suitable for inhaling deep into the lungs. When the GLP-1-loaded microspheres reach the lungs, FDKP dissolves rapidly at physiological pH and is excreted in its original form in the urine.⁶⁶ GLP-1 is then released and absorbed into the body circulation. In a study evaluating the safety, tolerability, pharmacokinetics, and pharmacodynamics of MKC253 at

different doses, 26 healthy subjects were randomized to receive a different dosage of MKC253, 12 of whom reported cough, which was mild and not dose-related, merely limited to the period before and after dosing. In addition, no subjects reported nausea, vomiting, or hypoglycemic reactions.⁶⁷ In an additional study assessing the safety and tolerability of MKC253, 20 patients with T2DM were randomly assigned to receive MKC253 or a placebo. (Technosphere inhalation powder without GLP-1). Cough accounted for 85% of adverse events and was mild, with all subjects having no significant hypoglycemic symptoms.⁶⁷ These findings indicate that MKC253 is safe and well-tolerated. However, the specific dose absorbed into the body varies widely among individuals, so the standard dosage of the inhalation and the safety of long-standing use remain to be addressed. Although inhalation agents of the GLP-1RAs have not yet been studied, GLP-1 inhalation agents represented by MKC253 offer the possibility of their successful development.

5.1.4 Implantable devices

Implantable devices may be an alternative to injections. The ITCA 650, developed by Intarcia Therapeutics, is a matchstick-sized GLP-1 implantable osmotic pump. With the application of a sterile technique, the ITCA is placed in the patient's abdominal area to provide subcutaneous delivery of exenatide continuously.⁶⁸ This delivery system allows proteins, peptides, antibody fragments, and other highly potent small molecules to remain stable at or above body temperature for three or more years without compliance issues. The ITCA 650 has a great therapeutic effect, showing a reduction in fasting glucose levels, HbA1c, and body weight when treated with different doses. In a study evaluating the safety and tolerability of different doses of ITCA 650 for the treatment of T2DM, the ITCA 650 was estimated as well-tolerated, with only minor local changes at the insertion site and transient nausea and vomiting, most common at the highest dose.⁶⁹ However, the product has an obvious drawback since the device demands surgical removal or replacement, which requires a procedure performed by trained medical personnel during an outpatient visit. Currently, the product is not approved by FDA.

5.2 New targets: from single targets to dual targets and combination drugs

5.2.1 GLP-1 R/GIPR dual target

Both glucose-dependent insulinotropic peptide (GIP) and GLP-1 are the main incretins in humans. GIP has the same function as GLP-1 in regulating glucose levels in T2DM patients, including stimulating insulin secretion and slowing gastric emptying though being less effective.¹²⁷ In addition, GIP exerts therapeutic benefits beyond its pancreatic effects by improving insulin sensitivity and lipid homeostasis in adipose tissue. Studies have shown that concomitant administration of GIP and GLP-1 can significantly increase insulin response and glucagon

regulation.125126 Accordingly, tirzepatide (LY3298176), a linear peptide molecule containing 39 amino acids based on the native sequence of GIP, is developed as a dual GIP and GLP-1 receptor agonist. The starting amino acid sequence is similar to the one of GIP, while the C-terminal ten amino acid sequence of exenatide is used as the C-terminal of tirzepatide. With a 20-carbon fatty acid side chain introduced at Lys20 to promote drug binding to albumin, the mean half-life of tirzepatide is extended to 116.7 h and approved to be used as a once-weekly formulation.

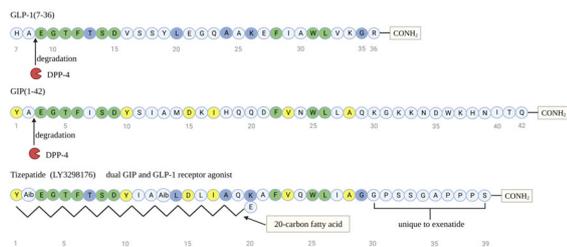


Figure 5. Schematic diagram of tirzepatide structure

A 26-week phase II clinical trial showed that tirzepatide was significantly more effective than dulaglutide in lowering blood glucose and reducing body weight.⁷¹ Frías JP et al. found that tirzepatide was superior to semaglutide in lowering glycated hemoglobin levels in T2DM patients and that the weight loss was approximately twice with tirzepatide treatment than with the once-weekly administration of the semaglutide group.⁷⁶

In the URPASS clinical trial, tripeptide at a high dose demonstrated fasting glycemic control compared to basal insulin preparations, a result previously achieved only with simulated among all GLP-1RAs.⁷³ It can be speculated that tirzepatide can better control postprandial glucose fluctuations compared to basal insulin.^{74,75} In addition, tirzepatide has the effect of lowering blood pressure, as well as increasing HDL (high-density lipoprotein) concentration and lowering VLDL (very low-density lipoprotein) and triglyceride concentrations, suggesting that it could be the priority for T2DM patients with combined hypertension or hyperlipidemia.⁷²

Furthermore, the pharmacokinetics of tirzepatide in patients with kidney damage had no significant changes compared to healthy subjects, indicating that dose adjustment may not be necessary for patients of this kind receiving tirzepatide.⁷⁷ Regarding drug safety, tirzepatide does not increase the risk of hypoglycemia but is associated with an elevated incidence of gastrointestinal reactions, the principal manifestation of which is nausea. The high doses lead to a significantly higher incidence of vomiting and diarrhea. Despite this, the overall incidence of adverse reactions in tirzepatide was similar to that in GLP-1RAs.⁷⁸ Currently, tirzepatide has been approved by the FDA, followed by its marketing application accepted by the CDE. It can be concluded that the GLP-1R/GIPR dual-target drug has a good development prospect.

5.2.2 Coadministration of GLP-1RA

One of the mechanisms by which GLP-1RAs lower blood glucose is to stimulate insulin secretion of β cells, but T2DM patients fail to achieve sufficient insulin secretion with severe exhaustion of β cells, thus reducing the efficacy of GLP-1RAs. Therefore, the combination of GLP-1RAs and basal insulin is a reasonable method for treating T2DM, and the drugs such as liraglutide/deguel insulin combination (Xultophy) and lixisenatide/glycine insulin combination (Soliqua) are both approved marketed drugs.

Sodium-glucose co-transporter protein-2 (SGLT-2) inhibitors, a new class of drugs for the treatment of T2DM, can suppress the reabsorption of glucose by the kidneys, allowing excess glucose to be excreted from the urine. Meanwhile, the drug also has the effects of lowering blood glucose, controlling body weight, and protecting β cells. It was found that the combination of GLP-1RAs and SGLT-2 has an additive effect on blood glucose control and does not cause hypoglycemia.⁷⁹ Interestingly, the drug has been associated with reduced risks of MACE, heart failure, renal failure, and other diseases⁸⁰, providing a more optimal treatment option for T2DM patients with cardiovascular or renal diseases.

Glucagon (amylin) is another hormone associated with hunger and satiety in addition to the GLP-1 signaling pathway, which mainly controls blood glucose and reduces body weight by inhibiting postprandial glucagon release secretion, delaying gastric emptying, and stimulating the satiety center to reduce energy intake. Novo Nordisk's CagriSema (a combination of semaglutide and the amylin analogue Cagrilintide) showed better effects of weight loss than a single drug in a phase II clinical study, with patients treated with CagriSema, semaglutide or Cagrilintide losing 2.18%, 1.79% and 0.93% of their body weight, respectively.¹¹² With semaglutide exerting a main hypoglycemic effect of the drug, the addition of Cagrilintide increases weight loss without side effects on the cardiovascular system, lungs, liver, or kidneys, making it an effective and safe treatment option. The drug received tacit approval for clinical trials in August 2022 and is intended for weight management in adult patients who are obese ($BMI \geq 30 \text{ kg/m}^2$) or overweight ($27 \text{ kg/m}^2 \leq BMI < 30 \text{ kg/m}^2$) with at least one weight-related complication, and for the adjunctive treatment with a reduced calorie diet and increased physical activity. However, further clinical studies are needed to determine whether CagriSema can be used for glycemic control in T2DM patients.

5.3 New application areas: from T2DM to multiple diseases

5.3.1 GLP-1RA and PCOS

GLP-1RAs can be used in the treatment of diseases other than T2DM. Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age. GLP-1RAs can improve hyperandrogenemia, ovulation, and menstrual disorders in women with PCOS

by reducing weight and increasing clinical pregnancy rates. [128] Bednarz K et al.⁸⁰ found a positive effect of GLP-1RAs on the overall status of patients with PCOS. This also accorded with an earlier observation, which showed that GLP-1RAs could play an essential role in the treatment of PCOS and in infertile women who are older or have an inadequate ovarian reserve,⁸² presumably related to the drugs' capabilities of increasing ovarian reserve and reducing the level of reactive oxygen species in the follicles of patients.

5.3.2 GLP-1RA and aging-related diseases

Research has demonstrated that GLP-1RAs can attenuate oxidative stress, delay cellular senescence⁸³ and exert neuroprotective and neurotrophic effects, facilitating neuronal cell protection, proliferation, and differentiation of precursor cells into neuronal cells.⁸⁴ The results of several clinical trials have shown that GLP-1RAs can be used to delay and treat age-related diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). GLP-1RAs could reduce the production and accumulation of the pathological marker of AD (β -amyloid) and attenuate the toxic reactions, [130] with the ability to reverse hyperphosphorylation of Tau protein in the brain and improve memory dysfunction. [129] In animal models of PD, GLP-1RA was found to reverse the decrease in tyrosine hydroxylase levels, increase the number of dopaminergic neurons, alleviate chronic inflammatory responses in the brain, and improve neuromotor dysfunction.⁸⁶ The results of experimental and epidemiological studies suggest that there is an association between T2DM and the occurrence of AD and PD, further indicating the advantages of using GLP-1RAs for T2DM in the treatment of aging-related diseases. [85][131][132]

5.3.3 GLP-1RA and COVID-19

The worldwide spread of novel coronavirus pneumonia (COVID-19) is seriously affecting human health, and many studies have shown a poor prognosis for COVID-19 in patients with T2DM. In a retrospective multicenter study of patients with T2DM and COVID-19, the authors reported that glycemic control was associated with significantly lower mortality.⁸⁷ The therapeutic role of GLP-1RAs for COVID-19 has not been clarified, but a national observational study in the United Kingdom found a neutral benefit of GLP-1RAs.

The study analyzed the risk of death in 2.85 million T2DM patients with COVID-19 and their glucose-lowering therapy before infection. Metformin, SGLT2i, and sulfonylureas are associated with a reduced risk of death for COVID-19, insulin and DPP-4i are associated with an increased risk, and GLP-1RA and thiazolidinedione are both neutral. It is speculated that this result may be related to the anti-inflammatory effects of GLP-1RAs, and the cardiovascular system and renal benefits could also play a part.⁸⁸ GLP-1RAs alone or in combination with metformin, for example, may be one of the treatment strategies for T2DM patients with comorbid

asymptomatic and non-severe COVID-19. However, more prospective studies are needed to assess whether these glucose-lowering injectable drugs in the context of COVID-19 are associated with better treatment outcomes and lower morbidity and mortality.

6. Summary and Outlook

GLP-1RA is a relatively safe and effective glucose-lowering drug, and recent studies have shown that GLP-1RAs can correct intestinal flora disorders while treating diabetes, effectively improving blood glucose control. [133] The combination of GLP-1RAs with probiotics can further improve glucose and lipid metabolism. [134] In addition, its weight loss effect and cardiovascular and renal benefits expand the scope of application. As research progresses, GLP-1RAs may become a therapeutic or adjunctive drug for diseases other than T2DM. It is foreseeable that the application prospect of GLP-1RAs will be highly extensive. Meanwhile, new formation of GLP-1RAs, for instance, oral drugs, powder nebulizers, and implantable devices, could solve the problems of injections, and the combination of GLP-1RAs with other hypoglycemic drugs or hormones is expected to enhance the therapeutic effects. In the future, targeted solutions to the defects of the new GLP-1RAs and more comprehensive studies on its potential adverse effects will play a vital role in the effective prevention and treatment of T2DM.

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