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Emerging technologies to achieve oral delivery of GLP-1 and GLP-1 analogs for treatment of type 2 diabetes mellitus (T2DM)

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Abstract

Glucagon-like peptide-1 (GLP-1) is a gastrointestinal (GI) peptide hormone that stimulates insulin secretion, gene expression and β-cell proliferation, representing a potentially novel and promising therapeutic agent for the treatment of T2DM. DPP-IV-resistant, long-acting GLP-1 analogs have already been approved by FDA as injectable drugs for treating patients with T2DM. Oral delivery of therapeutic peptides and proteins would be preferred owing to advantages of lower cost, ease of administration and greater patient adherence. However, oral delivery of proteins can be affected by rapid enzymatic degradation in the GI tract and poor penetration across the intestinal membrane, which may require amounts that exceed practical consideration. Various production strategies have been explored to overcome challenges associated with the oral delivery of therapeutic peptides and proteins. The goal of this review is to provide an overview of the current state of progress made towards the oral delivery of GLP-1 and its analogs in the treatment of T2DM, with special emphasis on the development of plant and food-grade bacterial delivery systems. Recently, genetically engineered plants and food-grade bacteria have been increasingly explored as novel carrier systems for the oral delivery of peptide and protein drugs. These have a largely unexplored potential to serve both as an expression system and as a delivery vehicle for clinically relevant, cost effective therapeutics. As such, they hold great promise for human biopharmaceuticals and novel therapies against various diseases.

Keywords: Oral drug delivery, GLP-1, GLP-1 analogs, Type 2 diabetes, Emerging technology, Genetically modified plants, Genetically modified food-grade bacteria

Introduction

Approximately 350 million people have diabetes worldwide which is estimated to double in the next 20 years [1]. Diabetes is a condition characterized by high blood sugar (glucose) levels, with Type II diabetes mellitus (T2DM) being the most common form, accounting for approximately 90–95% of all diabetes [2]. T2DM develops when the body becomes resistant to insulin or when the pancreas stops producing sufficient insulin for glucose control. This differs from the absolute loss of insulin secretion in Type 1 diabetes (T1D), which follows the autoimmune destruction of pancreatic beta cells. It is unknown why beta cell dysfunction and insulin resistance occurs, although genetics and environmental factors, such as excess weight and inactivity, seem to be the primary factors [3]. Current treatments for T2DM consist of non-pharmacological approaches such as diet and exercise and the pharmacological treatment (insulin and/or oral anti-hyperglycemic agents such as metformin and sulfonylureas). However, conventional drugs are often associated with undesirable side effects including weight gain and hypoglycemia. Moreover, many of them frequently exhibit reduced efficacy over time, leading to inadequate glycemic control [4]. Thus, there is an urgent need to develop safer and more effective alternatives. GLP-1-based therapies may offer a novel treatment option for T2DM, having the potential to overcome many of the limitations associated with conventional antidiabetic agents.

GLP-1 is a 30-amino acid gut peptide hormone produced in the intestinal epithelial endocrine L-cells. GLP-1 was initially discovered for its ability to increase the insulin secretion in response to nutrient ingestion, a phenomenon known as the 'incretin effect' [5]. Further research has demonstrated that GLP-1 reduces food intake (appetite

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suppression), slows gastric emptying, inhibits glucagon secretion, improves glucose homeostasis and insulin sensitivity, stimulates beta-cell regeneration and prevents beta-cell apoptosis (summarized in Fig. 1; also see [6, 7]). In patients with T2DM, GLP-1 secretion is reduced as compared to healthy subjects, thus making it a logical target for the development of new anti-diabetic therapeutics [5]. As the insulinotropic actions of GLP-1 are strictly glucose-dependent, its administration does not cause hypoglycemia, an unwanted side effect of common anti-diabetic drugs. Unfortunately, native GLP-1 has limited clinical utility due to its short circulating half-life (1–2 min), attributable to degradation by the ubiquitous enzyme, dipeptidyl peptidase IV (DPP-IV) [8]. To overcome the clinical limitations of native GLP-1, several DPP-IV-resistant longer-acting GLP-1 analogs, such as exenatide and liraglutide, have been developed and are approved by FDA to treat patients with T2DM. Exenatide (marketed as Byetta) is a synthetic version of exendin-4, a hormone found in the saliva of the Gila monster lizard [9]. Exendin-4, a 39-amino acid peptide, shares a 53% sequence homology with human GLP-1, is resistant to DPP-IV and has a circulating half-life of 2.4 h [10]. On the other hand, liraglutide (marketed as Victoza) is a chemically modified form of human GLP-1. It differs from GLP-1 by only one amino acid substitution as well as a fatty acid side chain, resulting in resistance to DPP-IV and a prolonged circulating half-life of 13 h [11]. To have an effect, both exenatide and liraglutide need to be given by subcutaneous injection, with exenatide twice daily and liraglutide once daily. Injections are often painful and disliked by patients, particularly if such agents are used in treating a chronic disease such as T2DM requiring ongoing treatment to maintain therapeutic benefit. This also makes the cost of treatment to be high.

Oral administration is often the most desirable and attractive choice for drugs due to convenience and patient adherence, especially when the drug of interest must be administered repeatedly [12]. However, the oral route for drug administration, especially for peptide and protein drugs constitutes a great challenge due to rapid degradation by digestive enzymes of the gastrointestinal (GI) tract and poor penetration across the intestinal epithelium into the blood circulation. Furthermore, the mucus layer on the epithelium provides a barrier for the diffusion of such macromolecules. These limitations often lead to the low bioavailability of most orally administered peptide and protein drugs [12, 13]. Some of this may be overcome by simply increasing the amount given, but for drugs that are expensive to produce; this strategy obviously is limited in practical terms. Various other strategies have been explored for enhancing the bioavailability of orally administered peptide and protein drugs, including chemical modifications of peptides or proteins to improve their stability, PEGylation to alter their physiochemical properties, co-administration of absorption enhancers, addition of other novel functions and the use of efficient delivery carriers [14]. Some of these strategies have been used successfully, for example, to achieve clinical applications of oral insulin delivery [15]. This review highlights the recent advances and progress made towards the oral delivery of GLP-1 and its analogs, with emphasis placed on developing genetically engineered plants and food-grade bacteria as new carrier systems for oral delivery of GLP-1. Plant and food-grade bacterium-based carrier systems, which combine expression and delivery of peptides or proteins in one system, are envisaged to offer an immense potential for the development of low-cost and easy-to-administer innovative biopharmaceutical products that are affordable,

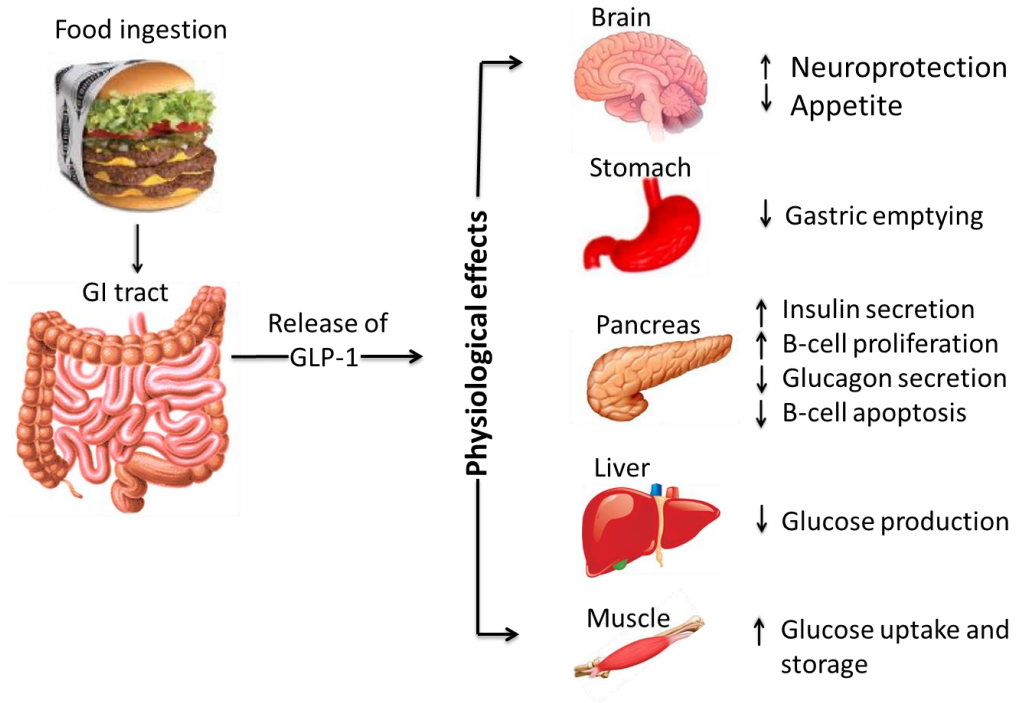

Fig. 1: GLP-1 is released from the small intestine into the circulation after food ingestion, and then exerts diverse biological actions on distinct target tissues, which lead to reduction of blood glucose level and body weight in humans. GLP-1 has a plasma half-life of less than 2 minutes, due to rapid inactivation by DPP-4 enzyme. One strategy is to use GLP-1 analogs that have a prolonged plasma half-life.

readily available and easily accessible.

Chemical modulation strategy

Chemical modification is a widely used tool to improve the bioavailability of therapeutic peptides and proteins while retaining their optimum pharmacological activity. The conjugation of polyethylene glycol (PEGylation) is a well-established method for extending the half-life and thus duration of action of bioactive molecules such as peptides and proteins [16, 17]. By conjugating PEG molecules to GLP-1, Youn et al. [18] demonstrated that the PEGylated GLP-1, compared to the unmodified native GLP-1, had a significantly longer circulating half-life and exhibited greater efficacy on glycemic control in diabetic mice after intranasal administration. Biotin is essential for the metabolism of proteins and carbohydrates, which is actively absorbed from the diet via a sodium-dependent transporter known as the sodium-dependent multivitamin transporter (SMVT) in the intestine [19]. In an attempt to increase the oral bioavailabilty of GLP-1 through increasing its stability and intestinal absorption, Chae et al. [20] conjugated both biotin and biotin-PEG to GLP-1. The resultant conjugates, biotin-GLP-1 (DB-GLP-1) and biotin-PEG-GLP-1 (DBP-GLP-1), showed 2.4- and 9.9-fold increase, respectively, in proteolytic stability compared with native GLP-1. Assessment of their effect on insulin secretion in isolated rat pancreatic islets revealed that both conjugates retained full insulinotropic activity of GLP-1. Importantly, while both DB-GLP-1 and DBP-GLP-1 displayed significantly improved glucose-lowering activity when compared with native GLP-1($p<0.001$) following oral administration to diabetic mice, DBP-GLP-1 had a more profound effect, resulting in a 24.5% reduction in 0–180 min AUC plasma glucose values compared with GLP-1 controls (Fig. 2). The superior glucose-lowering ability of DBP-GLP-1 is likely due to improved intestinal absorption, as the plasma concentration of GLP-1 was found to be markedly increased 30 min after oral administration of DBP-GLP-1. Similar results have also been reported for biotinylated exendin-4 analogs [21].

Use of polymer-based particulate delivery systems

Over the past two decades, large numbers of natural and synthetic biodegradable polymers have been utilized in the formulation of micro- and nanoparticle systems for targeted delivery of therapeutic molecules. The advantages of nanoparticulate drug delivery systems include protecting the drug molecules from enzymatic degradation, enhancing biopharmaceutical properties and providing passive or active targeting or sustained delivery [22]. Poly(lactic-co-glycolic acid) (PLGA), a FDA-approved synthetic polymer, is one of the most widely used polymeric biomaterials for developing nanodrug delivery systems for peptide and protein drugs owing to its good biocompatibility, biodegradability, nontoxicity and mechanical strength [23]. Joseph et al. [24] demonstrated that when encapsulated within PLGA and delivered orally in the form of GLP-1-PLGA-COOH nanoparticles, D-Ala2-GLP-1, a DPP-4-resistant analogue, lowered glycemic response in diabetic mice by 27% and 28% in plasma glucose AUC at 4 and 8 h tests, respectively, when compared with vehicle treated control mice. The authors also showed a

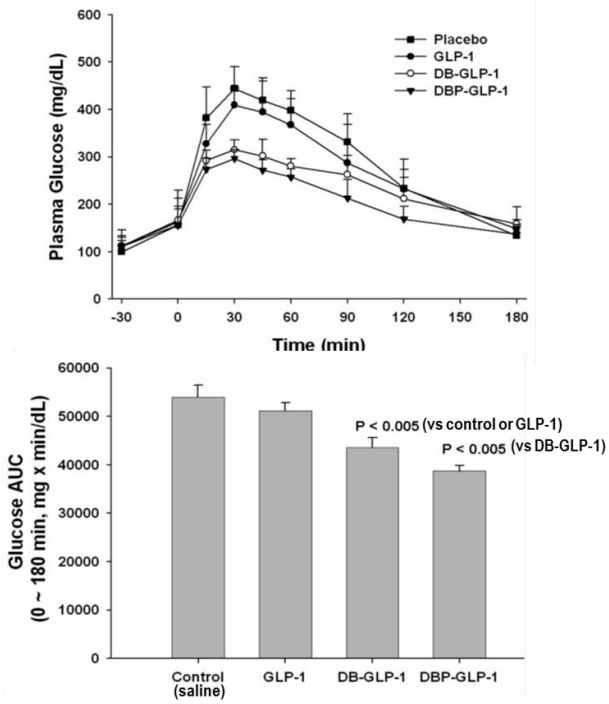

Fig. 2: Oral hypoglycemic efficacies of GLP-1, DB-GLP-1 and DBP-GLP-1 in diabetic mice. Reprinted from Bioconjugate Chemistry [20].

reduction in basal glycemia in GLP-1 nanoparticle treated mice by 23% and 35% at 4 and 8 h post treatment, respectively, compared with vehicle treated controls, suggesting that PLGA nanonoparticles can be used as an efficient oral delivery system for GLP-1.

Nguyen et al. [25] developed an oral formulation of exendin-4 consisting of pH-sensitive nanoparticles made from chitosan and poly-(γ-glutamic acid) coated with an enteric polymer. Chitosan, a linear carbohydrate polymer, has special features of adhering to the mucus layer and transiently opening tight junctions between epithelial cells, thus increasing the paracellular permeation of peptide drugs [26]. Poly-(γ-glutamic acid) is a natural biopolymer which is water-soluble, biodegradable and nontoxic [27]. The enteric coating remains intact in the acidic environment of the stomach (pH 1.5 to 3.5), protecting the nanoparticles from disintegration during transit from the stomach. The coating dissolves in the higher pH (above pH 5.5) of the small intestine, releasing exendin-4 loaded nanoparticles. Once in the small intestine, these nanoparticles become less stable and disintegrate due to their pH sensitivity, releasing the encapsulated peptide which then permeates through the opened paracellular pathway into the systemic circulation. Indeed, the authors found that maximum plasma concentration of exendin-4 was reached in 5 h after oral administration of exendin-4 loaded nanoparticles in rats. As expected, orally administered exendin-4 loaded nanoparticles stimulated insulin release and had a prolonged duration of glucose-lowering action. No plasma exenatide (synthetic exendin-4) was detected in the control group receiving oral blank nanoparticles blended with free exenatide. Recently, Zhang et al. [28] reported a pH-responsive microsphere system composed of alginate and hyaluronate for the oral delivery of exendin-4. Alginate and hyaluronate are carbohydrate polymers that are known to shrink at low pH of gastric environment, preventing release of the encapsulated drugs in the stomach [29]. The authors showed that exendin-4 reached its maximum plasma concentration in 4 h after oral administration of the microspheres in mice. Further, the treatment reduced blood glucose to a normal level within 2 h after the oral intake and it was maintained for another 4 hours.

Genetically engineered plants as delivery systems

Plants are the main food source for most living things, including humans. Plant-based foods can provide nearly all the nutrients (vitamins, minerals, fiber, proteins and phytochemicals) that the body requires. Recent advances in plant biotechnology have made it possible to add new roles to plants, i.e. plants can now be used as green bioreactor systems for the production of recombinant proteins [30]. Plants as a protein production platform offer considerable advantages over conventional fermentation-based cell culture expression systems, including the ease and low cost in generating a large biomass, easy and inexpensive to scale up (increase of planted acreage), natural storage organs (tubers, seeds), low risk of product contamination by animal or human pathogens (HIV, hepatitis B virus), the ability of plant cells to perform complex post-translational modifications required for proper folding and functionality of many proteins, and the availability of established practices for efficient harvesting, transporting, storing and processing of plant material [30, 31]. Furthermore, production of therapeutic peptides and proteins in food crops provides the advantages of direct delivery through ingestion of edible transformed plant tissues without the need for an expensive purification process. Unlike animal cells, plant cells have a thick, outer cell wall primarily made of cellulose microfibrils that are chemically stable and resistant to enzymatic digestion. Upon oral delivery, peptide or protein drugs bioencapsulated in plant cells are protected in the stomach from acids and enzymes but are subsequently released into the gut lumen by microbes that digest the plant cell wall [30, 32]. Additionally, since many plants, ranging from non-food crops such as tobacco to food and vegetable crops such as soybean, rice and tomato, can be genetically transformed and regenerated using *Agrobacterium*-mediated genetic transformation or direct gene transfer methods (Fig. 3), this would allow greater flexibility for researchers to choose the most appropriate plant host to express and deliver the therapeutic agents, making the plant-based oral therapy more palatable [32].

In recent years, there has been increasing use of plants to produce high-value biotherapeutic agents such as cytokines, enzymes, edible vaccines and antibodies [30, 31, 33]. The carrot cell-derived human glucocerebrosidase enzyme (GCB) has been approved by FDA and maketed under the trade name Elelyso™, for the treatment of patients with Gaucher disease [34]. Plant-derived vaccine candidates against various influenza strains (e.g. H5N and H1N1) have been tested in phase I and phase II human clinical trials and were found to be safe and well tolerated [35]. The recent demonstration that genetically engineered plants can be used to express and deliver GLP-1 analogs in the treatment of experimental T2DM offers plant-based new, low-cost treatment possibilities for human T2DM.

Delivery of GLP-1 analogs via non-food plants

Tobacco (*Nicotiana tabacum*), despite a traditionally negative view due to its ties to smoking, is one of the most widely used plant hosts for the production of pharmaceutical proteins. As a green bioreactor, tobacco offers unique advantages over other plant species such as high biomass yields and the suitability for genetic engineering. Further, the availability of low nicotine, low-alkaloid tobacco varieties has made tobacco plants even suitable for direct oral delivery of biopharmaceutical products in plant material or crude extracts [30]. To establish a proof-of-concept for use of plants for GLP-1 production, Brandsma et al. [36] generated genetically engineered (transgenic) tobacco plants containing a synthetic gene encoding a large multimeric form of GLP-1 analog composed of ten tandem repeats of the GLP-1 sequence (GLP-1x10) in order to achieve high-level accumulation of the peptide in plant cells. Western blot analysis showed high-level accumulation of GLP-1x10 analog of the expected size in tobacco leaves. Functional analysis of partially purified GLP-1x10 analog using a mouse pancreatic beta cell line (MIN6) showed that plant-derived GLP-1x10 analog retained its ability to stimulate glucose-dependent insulin secretion, suggesting the feasibility of transgenic plants as an expression host for the production of functional GLP-1 analogs.

Subsequently, the same research group [37] generated transgenic tobacco plants expressing exendin-4 (Ex-4) fused to human transferrin (Tf). Tf is a naturally occurring iron transport protein used by the body to transport iron into the bloodstream through a receptor mediated endocytotic process [38]. The Tf receptor is abundantly expressed in the human gastrointestinal epithelium and, because Tf is relatively resistant to proteolysis in the GI tract, it has the potential to facilitate the delivery of oral drugs across the intestinal epithelium. The authors demonstrated that plant-made Ex-4-Tf fusion protein retained the insulinotropic activity of exendin-4 as well as transferrin's ability to internalize into human intestinal cells.

Importantly, oral delivery of partially purified plant-derived Ex-4-Tf significantly improved glucose tolerance, whereas oral delivery of free Ex-4 had no effect in mice (Fig. 4). Moreover, these authors showed that tobacco plants provide a robust system for the production of Ex-4-Tf, producing up to 37 µg Ex-4-Tf/g fresh leaf tissue.

Kwon et al. [39] generated chloroplast-transformed transgenic (transplastomic) tobacco plants for the oral delivery of exendin-4 fused to cholera toxin B subunit (CTB-Ex-4). CTB is recognized as a valuable transmucosal carrier that facilitates transportation of the conjugated proteins into circulation by binding to GM1 receptors

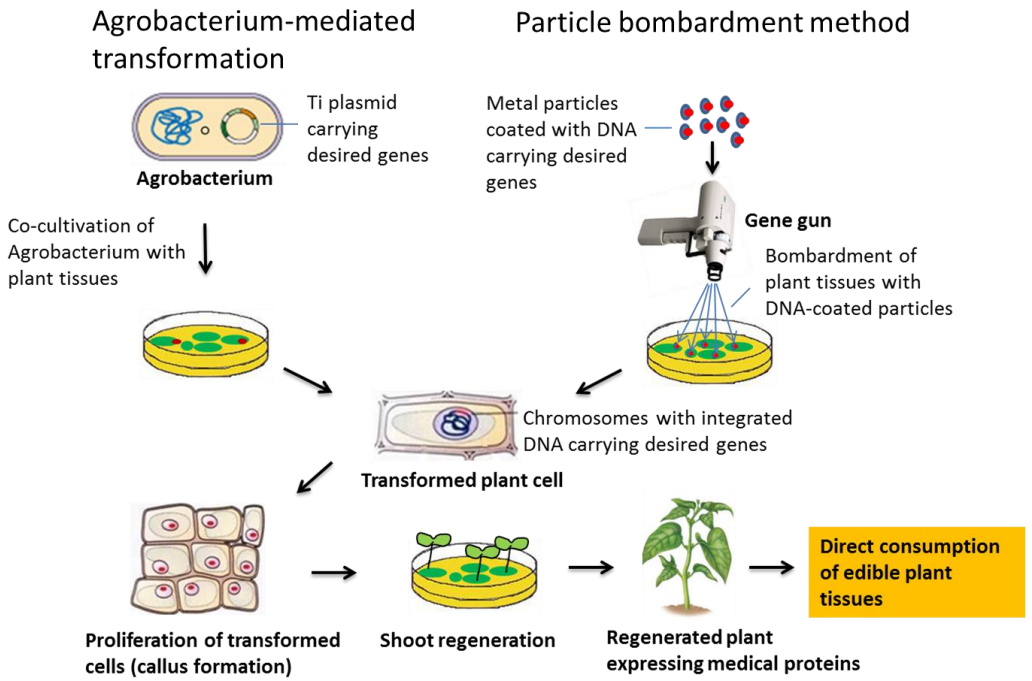

Fig. 3: Schematic diagram showing major steps involved in the generation of transgenic plants using *Agrobacterium*-mediated transformation and biolistic bombardment (gene gun) methods. Reprinted with permission from Current Pharmaceutical Biotechnology

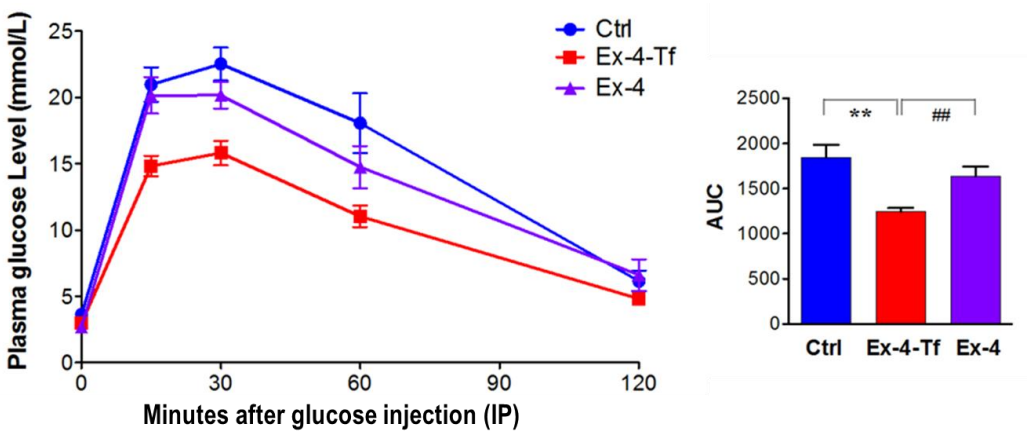

Fig. 4: Effect of oral administration of plant-made Ex-4-Tf fusion protein on plasma glucose tolerance in mice. Intraperitoneal glucose tolerance tests (IPGTT) was carried out in mice treated with sterile saline (Ctrl), partially purified Ex-4-Tf (prEx-4-Tf) or commercial Ex-4 (Ex-4) (n=5-6 in each group). Glucose responsiveness of the corresponding experimental groups is shown as a measurement of AUC of the IPGTT graphs with units of mM/min. Data are expressed as means ± SEM. ** $P < 0.01$ (One-way ANOVA) and ## $P < 0.05$ (unpaired Student's t-test) versus the ctrl group. Reprinted with permission from Plant Biotechnology Journal [37].

present on the surface of intestinal mucosal cells [40]. A major advantage of producing foreign proteins in the transformed chloroplasts of higher plants is high protein yields [41]. CTB-Ex-4 expression levels reached up to 14.3% of total leaf protein. Lyophilization of leaf material increased therapeutic protein concentration by 12–24 folds, extended their shelf life up to 15 months when stored at room temperature. The pentameric structure, disulfide bonds and functionality of CTB-Ex-4 were well preserved in lyophilized materials. Results from *in vitro* functional analysis using mouse-derived pancreatic beta-TC-6 cells showed that chloroplast-derived CTB-Ex-4 stimulated glucose-dependent insulin secretion to the level similar to commercial Ex-4. Oral administration of lyophilized CTB-Ex-4 expressing leaf tissues stimulated insulin secretion similar to the intraperitoneal injection of commercial Ex-4 in mice.

Delivery of GLP-1 analogs via vegetable plants

Zhao et al. [42] generated transgenic cucumber synthesizing a fusion protein consisting of ten tandem repeats of the GLP-1 sequence. Compared to the use of tobacco plants as a delivery system, fruits and vegetables are more attractive because they are palatable and can be eaten raw. Western blot analysis showed stable expression of fusion protein of the expected size in transgenic cucumber. Oral administration of a partially purified preparation of GLP-1 fusion protein significantly lowered blood sugar levels in diabetic rats.

Genetically engineered food-grade bacteria as delivery systems

Lactic acid bacteria (LAB) are a heterogeneous group of non-sporulating gram-positive bacteria that produce lactic acid as their major fermented product. LAB have a long history of use by humans for food production and food preservation, and have earned Generally Recognized as Safe (GRAS) status [43]. LAB can be found in the human GI tract, where they constitute a regular component of the normal microbial flora playing important roles in host gut health (anti-inflammatory, anti-infective and immunomodulatory properties), so called "helpful" bacteria or probiotics [44, 45]. Most of the currently used LAM strains for food products have been isolated from the intestinal microbiota of healthy humans, which are known to have a strong beneficial relationship between the bacterium and its human host. Among them, *Lactobacillus* and *Bifidobacterium* are the most frequently used species [45]. Due to these favorable characteristics, commensal or food-fermenting LAB have recently been increasingly investigated for use as live mucosal vectors for the production and *in situ* delivery of biopharmaceuticals, especially peptide and protein drugs [45, 46]. The administration of such recombinant microorganisms would significantly lower the production cost of the treatment agents since being live organisms, such vectors would be able to autonomously amplify, produce and deliver the product of interest [46, 47]. Thus far, recombinant *L. lactis* has been successfully used to target the delivery of anti-inflammatory cytokine IL-10 to intestinal mucosa for the treatment of experimental inflammatory bowel disease [48], and was further investigated in a Phase I study in patients with crohn's disease [49]. *L. lactis* (AG013) has also been modified to secrete trefoil factor 1 for the treatment of oral mucositis, and is currently in Phase Ib clinical trials [50]. Mucosal delivery of recombinant *L. lactis* MG1363 secreting the diabetic autoantigen proinsulin or glutamic acid decarboxylase (GAD 65) along with IL-10 reversed T1D in NOD (non obese diabetic) mice [51, 52], while oral vaccination with recombinant *L. plantarum* NCL21 expressing a common Japanese cedar pollen allergen, Cry j 1(Cry j 1-LAB), ameliorated the symptoms of cedar pollinosis and decreased allergen-specific IgE production in a murine model of cedar pollinosis [53]. A recent progress made in the application of LAB as live mucosal vectors is the production and delivery of GLP-1 and GLP-1analogs for the treatment of T2DM.

Delivery of GLP-1 analogs using probiotic Lactobacillus strains

Duan et al. [54] constructed a recombinant strain of *Lactobacillus gasseri* for the expression and delivery of inactive full-length form of GLP-1(1-37) for treating T1D. The work was based on their previous study demonstrating that human commensal strain *Escherichia coli* Nissle 1917 could be engineered to express and deliver GLP-1(1-37) to human intestinal carcinomas and stimulate glucose-responsive insulin secretion [55]. *L. gasseri* is a commensal, lactic acid-producing bacterium of the human GI tract and is a GRAS-status organism. In humans, *L. gasseri* elicits various health benefits through its antimicrobial activity, bacteriocin production, and immunomodulation of the innate and adaptive systems [56]. It is also the most commonly used bacterial strain for probiotic purposes [57]. In this study, the authors found that type 1 diabetic rats fed GLP-1(1-37)–secreting *L. gasseri* cells daily had significantly reduced blood glucose levels and increased insulin levels and, additionally, were significantly more glucose tolerant than those fed the parent bacterial cells. Furthermore, treated animals developed insulin-producing cells within the upper intestine in numbers sufficient to replace ∼25–33% of the insulin capacity of nondiabetic healthy rats (Fig. 5), suggesting that feeding engineered commensal bacteria could potentially reprogram intestinal epithelial cells into insulin-producing cells. These results provide evidence of the potential of genetically engineered LAB for use as a safe and effective oral treatment for diabetes.

Zeng et al. [58] reported the generation of an engineered commensal strain of *L. paracasei* for the oral delivery of exendin-4. In this work, they showed that the engineered bacterial strain was able to produce and secrete recombinant exendin-4. Like GLP-1, the secreted exendin-4 significantly enhanced insulin secretion and promoted cell proliferation and survival in INS-1 β-cells. Permeability assessment using *in vitro* Caco-2 cell monolayer model revealed a 34-fold increase in the transport of exendin-4 across Caco-2 cells when delivered by *L. paracasei* cells compared with free exendin-4 transport, suggesting the effectiveness and efficiency of this bacterial delivery system in increasing exendin-4 transport across the intestinal barrier.

Delivery of GLP-1 analogs using probiotic Bifidobacterium strains

Wei et al. [59] reported the construction of a recombinant strain of *Bifidobacterium longum* for use as a live bacterial vector to deliver GLP-1 fused to penetratin. Penetratin is a well-characterized cell-penetrating peptide known to enhance cellular uptake of peptide/protein cargo [60]. *B. longum* is one of the most abundant species of bacteria in the human GI tract and is also one of the most popular probiotics. It has particular health promoting properties for GI and immune health, including promoting healthy cytokine

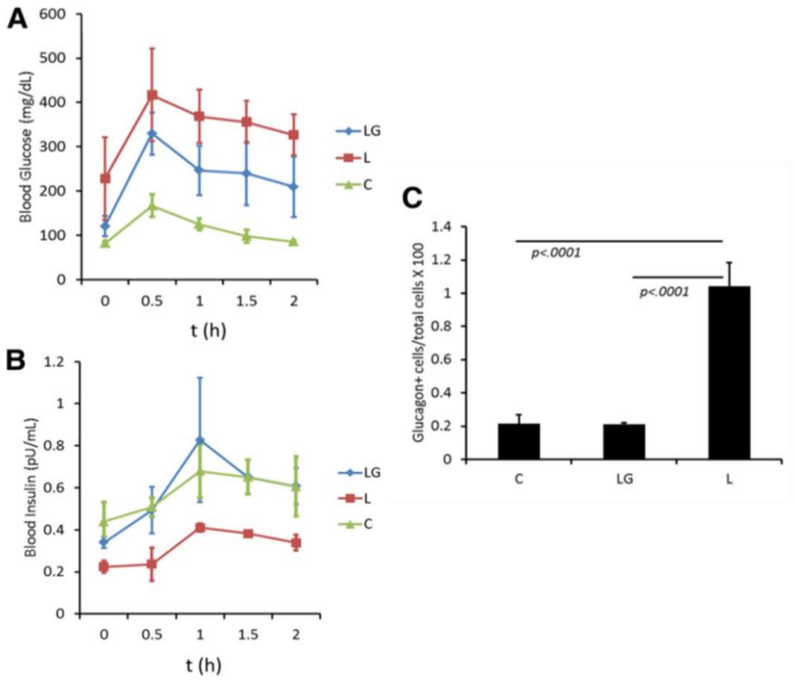

Fig. 5: Effect of bacterial feeding on hyperglycemia in STZ induced diabetic rats. After treatment with *L. gasseri* (L) and *L. gasseri*-secreting GLP-1(1-37) (LG), rats were subjected to an OGTT. Control rats (C) were not treated with STZ and fed sterile media. Blood glucose **(A)** and insulin **(B)** levels were measured for the OGTT. Data are expressed as means ± SEM (n = 6). **(C)** Pancreatic sections from each treatment group were morphometrically analyzed for the ratio of glucagon-positive cells to total pancreatic cells. Reprinted from Diabetes (54).

production in the colon and maintaining healthy GI barrier function, in part by supporting the mucosal lining of the GI tract [61]. In this report, the authors showed that penetratin-GLP-1 fusion protein secreted by the bacterial transformants retained the function of natural GLP-1. Moreover, the results of *in situ* intestinal absorption experiments indicate that the fusion protein is well absorbed in the small intestine and colon.

Clinical Results

Novo Nordisk (Plainsboro, NJ, USA), has developed an oral tablet formulation of the long-acting GLP-1 analogue, known as semaglutide, using the Eligen™ oral drug delivery system developed by Emisphere Technologies, Inc (Roseland, NJ, USA). Eligen™ oral drug delivery platform is based on the use of synthetic nonacylated amino acids as carriers, which allows the drug molecules to enter the circulation through the body's natural passive transcellular transport processes in the small intestine. Novo Nordisk completed a phase II clinical trial of orally administered semaglutide in patients with early T2DM. The results show that the oral administration of semaglutide is effective in lowering blood sugar levels and reducing body weight in a dose-dependent manner [62]. Oral semaglutide is now being studied in a large phase 3 clinical trial. In addition to semaglutide, Novo Nordisk, partnered with Merrion Pharmaceuticals (Dublin, Ireland), developed another oral GLP-1 analog (NN9928) using Merrion's proprietary GIPET (gastrointestinal permeation enhancement technology) drug delivery platform. The GIPET technology uses small- and large-molecule surface active materials (fatty acids and derivatives, surfactants and lecithin) to enhance drug absorption in the small intestine. Phase I trials yielded promising results and phase II trials are planned [63].

Oramed Pharmaceuticals (Jerusalem, Israel) developed an oral version of exenatide (ORMD 0901) using the company's proprietary POD™ (protein oral delivery) technology. The POD technology employs protectants in combination with an absorption enhancer to prevent degradation of the peptide in the GI tract and enhance its absorption. In a small scale preliminary proof of concept study, ORMD-0901 demonstrated an excellent glucose reduction efficacy in both animals (dogs) and human healthy volunteers [64].

There are also other companies that are actively involved in developing oral formulations of GLP-1, although they still remain in pre-clinical phases. For example, Diabetology Ltd (Jersey, UK) is using its Axcess™ oral drug delivery system to develop an oral GLP-1 analog. The Axcess™ delivery system is based on an enteric-coated capsule filled with the drug and absorption and solubility enhancers designed to increase the absorption of peptides, proteins and other macromolecules across the intestinal wall when delivered orally without any chemical modification of the active compounds [65]. Biolaxy (Shanghai, China) is developing an oral exenatide, Nodexen, using the NOD (nanoparticle oral delivery) technology platform [65].

Conclusion

Glucagon-like peptide-1 and its analogs represent an emerging new class of antidiabetic drugs that offer many clinical benefits over existing therapies for the treatment of T2DM. They effectively reduce

blood glucose levels, augment insulin secretion and preserve β-cell function, inhibit glucagon secretion, promote weight loss, delay gastric emptying and reduce the risk of diabetic complications. Currently, all GLP-1 analogs approved for clinical use need to be administered by injections using needles. However, the oral route is the most desirable for their delivery although this route of drug delivery still remains challenging.

A growing number of approaches have been developed to tackle the challenges associated with the oral delivery of peptide and protein drugs. As discussed in this review, advances in the oral delivery of GLP-1 and its analogs have been made by the use of absorption enhancers and direct structural modification of the peptide. Polymeric nanoparticle-based delivery systems have been utilized to increase their stability and intestinal absorption. Several pharmaceutical companies have developed unique oral formulations of GLP-1 based on their patented delivery system that have already progressed into clinical trials. Genetically engineered plants and food-grade bacteria, being emerging oral drug delivery systems, have demonstrated their competence in oral GLP-1 delivery. Plant- and food-grade bacterium-based delivery systems offer several potential advantages over more traditional drug delivery methods such as chemical modification of drugs, including decreased cost and enhanced convenience. One major concern with the use of LAB as live mucosal vectors is the possibility of uncontrolled proliferation and release into the environment. To address this safety issue, autotropic *L. lactis* strain was created, requiring thymidine to grow. Without thymidine, it cannot survive in the environment and containment was validated *in vivo* in pigs [66]. To make oral GLP-1 and its analogs to become a realistic therapy, many more *in vivo* and human studies are necessary. Importantly however, as outlined in this review, there are good reasons to believe that oral forms of GLP-1 and its analogs will likely play an important part in the management of human T2DM in the near future.

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Conflict of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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