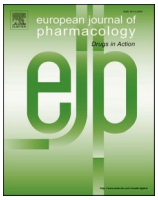
European Journal of Pharmacology 928 (2022) 175107

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar



GZR18, a novel long-acting GLP-1 analog, demonstrated positive *in vitro* and *in vivo* pharmacokinetic and pharmacodynamic characteristics in animal models

Man Zhang [a], Yining Zhang [a], Xiaohong Peng [c], Anshun He [a], Yue Wang [b], Ying Deng [a], Cheng Cui [a], Fangkai Xue [a], Bing Wei [a], Wancai Xing [a], Yuzhen Qian [a], Michelle Mazuranic [b], Wei Chen [a,*]

[a] *Gan & Lee Pharmaceuticals, Beijing, China*
[b] *Gan & Lee Pharmaceuticals USA Corp., Bridgewater, NJ, USA*
[c] *State Key Laboratory of Membrane Biology, Beijing Key Laboratory of Cardiometabolic Molecular Medicine, Institute of Molecular Medicine, Peking University, Beijing, China*

ARTICLE INFO

Keywords:
GZR18
GLP-1 analog
GLP-1 receptor agonist
Type 2 diabetes
Pharmacokinetics
Pharmacology

ABSTRACT

GZR18 is a novel analog of glucagon-like peptide-1 (GLP-1). This study evaluates the pharmacology, pharmacokinetics, and efficacy of GZR18, and its potential for the treatment of Type 2 diabetes mellitus (T2DM). *In vitro* pharmacology and activity of GZR18 were characterized by a binding assay of GZR18 using human serum albumin (HSA), an activation assay in human GLP-1 receptor-expressing cell lines, and its effect on glucose-stimulated insulin secretion (GSIS) in primary mice islets. Pharmacokinetic profiling was performed in Sprague Dawley rats and cynomolgus monkeys, and efficacy evaluated using GZR18 single or repeated doses in db/db mice. GZR18 showed similar binding affinity for HSA and GLP-1 receptor compared with semaglutide and liraglutide. GZR18 increased GSIS, which was confirmed by dynamic islet perifusion and fluorescence imaging using PKZnR-5 for real-time detection. In cynomolgus monkeys, the average GZR18 maximal concentration was 527 nmol L^{-1}, the terminal half-life ($T_{1/2}$) was 61.3 h, and the time to maximum concentration was 14 h. Single-dose GZR18 lowered blood glucose levels and reduced body weight over 72 h in db/db mice. GZR18 successive administration (every three days for 33 days, i.e. 11 doses) lowered nonfasting and fasting blood glucose levels ($p < 0.05$ versus control) and glycated hemoglobin, following the 11th dose. Food and water consumption in db/db mice was lowered following repeated doses of GZR18 ($p < 0.05$ versus control), without a reduction in body weight. These results demonstrate the potential of GZR18 as a long-acting GLP-1 analog for the treatment of T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder, characterized by hyperglycemia, which, if left untreated, can lead to many complications, such as cardiovascular disease, neuropathy, and nephropathy (Zheng et al., 2018). The binding of the incretin hormone, glucagon-like peptide-1 (GLP-1), to GLP-1 receptors triggers the release of insulin from β-cells and suppresses glucagon secretion (Andersen et al., 2018; Sharma et al., 2018). This glucose homeostasis is severely compromised in patients with T2DM which, in part, is due to reduced levels of GLP-1 (Nauck and Meier, 2016; Reed et al., 2020).

GLP-1 has a short half-life of approximately 1–2 min following intravenous (i.v.) administration, owing to its rapid degradation by the dipeptidyl peptidase-4 (DPP-4) enzyme, thus limiting its clinical application (Andersen et al., 2018). One of the widely used strategies to extend the half-life of peptide analogs is to increase their binding affinities to human serum albumin (HSA), which involves conjugation of GLP-1 with the albumin-binding domain (Strohl, 2015).

Several glucagon-like peptide-1 (GLP-1) receptor agonists have been marketed, including exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, and semaglutide (Chun and Butts, 2020). GLP-1 receptor agonists can be categorized according to their duration of action:

* Corresponding author. No. 8 Nanfeng West 1st Street, Huoxian, Tongzhou District, Beijing, 101109, China.
E-mail address: wei.chen@ganlee.com (W. Chen).

https://doi.org/10.1016/j.ejphar.2022.175107
Received 10 February 2022; Received in revised form 6 June 2022; Accepted 13 June 2022
Available online 16 June 2022

short-acting (administered once or twice daily) and long-acting (administered once weekly). Long-acting GLP-1 receptor agonists have the advantage of reduced dosing frequency, ease of use, and better safety profiles, compared with once- or twice-daily GLP-1 receptor agonists, besides having robust glycemic effects (Cornell, 2020).

GLP-1 receptor agonists improve glycemic control, mainly by activating the GLP-1 receptors in the pancreas, stimulating insulin release from β-cells, and suppressing glucagon secretion from α cells, which results in blood-glucose-lowering in patients with hyperglycemia. GLP-1 receptor agonists are also known to facilitate weight loss by delaying gastric emptying and promoting satiety, with lower hypoglycemia risk compared with secretagogue agents (e.g. insulin) (Cornell, 2020; Sheahan et al., 2020). Owing to differences in their molecular size, structure, and pharmacokinetics, all GLP-1 receptor agonists differ in pharmacodynamics and clinical effects (Cornell, 2020). These differences highlight the importance of understanding the benefits and limitations of each GLP-1 receptor agonist when considering this drug class as a treatment option for patients with T2DM, to ensure optimal therapeutic outcomes.

GZR18 is a novel GLP-1 analog. Higher binding affinity to HSA is designed to be achieved via structural modification of a longer fatty acid chain at the side chain position, resulting in an extended duration of efficacy, owing to prolonged absorption and delayed elimination, and improved stability against degradation by DPP-4 enzymes. This study aimed to evaluate the pharmacology of GZR18 in GLP-1 receptor-expressing cell lines and primary mice islets, the pharmacokinetics and efficacy of GZR18 in animal models, and its potential for the management of T2DM.

2. Materials and methods

2.1. In vitro pharmacology and activity

2.1.1. Binding affinity study of GZR18 with GLP-1 receptor

Binding competition assays of ^{125}I-GLP-1 (PerkinElmer, Waltham, MA, USA) in the presence of low (0.005%) and high (2%) concentrations of HSA were performed as described in Lau J, et al., with minor modifications (Lau et al., 2015). The binding assay was performed using GLP-1 receptor membrane proteins (GenBank accession number NM_002062.4) which were extracted and purified by WuXi AppTec New Drug Development Co., Ltd (Shanghai, China) from a human GLP-1 receptor-expressing HEK293 cell line. Different concentrations of GZR18 (MW: 4139.15 Da) (Gan et al., 2021), liraglutide, and semaglutide were analyzed for their *in vitro* binding affinities to GLP-1 receptor in the presence of competitor ^{125}I-GLP-1. A total of 10 concentration gradients (500, 125, 31.25, 7.8125, 1.9531, 0.4883, 0.1221, 0.0305, 0.0076, and 0.0019 nM) were obtained by four-fold serial dilutions of GZR18, liraglutide, and semaglutide, and added to a 96-well plate. The GLP-1 receptor membrane proteins were added to make a final concentration of 2 μg/well; ^{125}I-GLP-1 was added to make a final concentration of 150 ρM. The 96-well plate was sealed and incubated for 1 h at room temperature. A GF/C filter plate (Unifilter-96 GF/C filter plates, PerkinElmer, Waltham, MA, USA) was incubated for 30 min in soaking buffer (50 mM N-2-hydroxyethyl piperazine-N-ethanesulfonic acid [HEPES], 0.5% bovine serum albumin [BSA], pH 7.4) prior to transferring the incubation solution from the 96-well plate to the GF/C filter plate. After the transfer, the GF/C filter plate was washed six times with washing buffer (50 mM HEPES, 5 mM ethylene glycol tetraacetic acid, 5 mM $MgCl_2$, 0.005% Tween-20, pH 7.4), followed by 1 h of drying at 50 °C. Scintillation fluid (50 μL) was added to each well, and radioisotope scintillation readings were obtained using a Microbeta reader. Each compound was individually tested three times. The 'log (inhibitor versus response) – variable slope' model was applied using GraphPad Prism (version 5.0) to fit and calculate the half-maximal inhibitory concentration (IC_{50}).

2.1.2. GZR18 binding to HSA

HSA in concentrations of 0.005% and 2% were used to assess the *in vitro* HSA-binding properties of GZR18, GLP-1 (7—37) (Haoyuan Instrument Co., Ltd, Dandong City, China), liraglutide, and semaglutide. The experiments were performed as described in Lau J, et al., with minor modifications (Lau et al., 2015). A CHO-K1-GLP-1 receptor cell line expressing both the human GLP-1 receptor and luciferase reporter gene (Gan & Lee Pharmaceuticals) was used for the biological activity assay in combination with the Bright-Glo Luciferase Assay (Promega, Madison, WI, USA) system. The plate was read using the luminescence detected with a Spark Microplate Reader (Tecan, Männedorf, Switzerland). Each sample was tested three times in duplicates, and data were analyzed using GraphPad Prism (version 6.0), to create concentration–response curves. The ratio of the concentration for 50% of the maximal effect (EC_{50}) for each drug was determined using the following equation:

$$\text{Ratio}\frac{2}{0.005}\%\ \ \text{HSA} = \frac{EC_{50}(2\%\ \ \text{HSA})}{EC_{50}(0.005\%\ \ \text{HSA})}$$

2.1.3. The effect of GZR18 on glucose-dependent stimulated insulin secretion (GSIS)

Pancreatic islets were isolated from 50 C57BL/6N male mice (6–7 weeks old) after being fasted overnight and sacrificed by cervical dislocation. Pancreases were extracted from the animals and incubated at 37 °C in the presence of collagenase for 15–17 min by intermittent shaking. The samples were placed on ice with twice the volume of Hank's balanced salt solution added, after which the samples were centrifuged at 4 °C at 350 g for 2 min – the centrifugation step was repeated three times. The supernatant was discarded, and islets were resuspended and cultured overnight in RPMI-1640 medium (11.1 mmol/L glucose and 10% fetus bovine serum). The next day, the pancreatic islets were selected under the microscope. In each experiment, the pancreatic islets were divided into five groups (low-glucose stimulation [2.8 mM glucose], high-glucose stimulation [11.2 mM glucose], 30 nM semaglutide [11.2 mM glucose], 30 nM GZR18 [11.2 mM glucose], 90 nM GZR18 [11.2 nM glucose]), each with 15–20 islets. The cells were starved for 30–60 min at 37 °C by adding glucose-free Krebs-Ringer bicarbonate-HEPES buffered (KRBH, 0.1% BSA) medium before adding KRBH medium containing 0.1% BSA and 2.8 mM glucose or 11.2 mM glucose, and semaglutide, or GZR18 in the different concentrations. The groups containing 11.2 mM glucose were subjected to glucose stimulation for 1 h at 37 °C, and the incubation solution was collected by centrifugation (1000 rpm, 2 min, 37 °C). Each experiment was repeated three times. The insulin content in the incubation solution was detected by fluorescence resonance energy transfer (FRET; 62IN2PEG, Cisbio Bioassays, Codolet, France) at an absorbance of 620 nm and 665 nm, and the average absorbance values and standard errors of the mean of the three experiments were calculated by three independent tests (three duplicates per test), using one-way ANOVA to detect significance. Islet function was deemed adequate if insulin release occurred more than twice following high and low glucose stimulation in the KRBH medium.

2.1.4. Islet perifusion, insulin secretion, and islet imaging

Overnight cultured and similar-sized pancreatic islets from several C57BL/6N male mice (10–13 weeks old) were selected and randomly assigned into three groups (control, semaglutide, and GZR18; 50 islets per group). After pre-incubation in Krebs-Ringer bicarbonate buffer (KRBB) solution (125 mM NaCl, 5.9 mM KCl, 2.56 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1 mM L-glutamine, 25 mM HEPES, 0.01% BSA, 3 mM glucose) for 2 h at 37 °C, islets were manually picked using a 20 μL micropipette and gently injected into the temperature-controlled perifusion chambers (infusion set filter chamber). The loaded islets were then perifused by a continuous flow of KRBB solution containing 3 mM glucose at 37 °C (pH 7.4) for 10 min. Then, KRBB containing different stimulators (11.1 mM

glucose [control], 11.1 mM glucose with 60 nM GZR18, 11.1 mM glucose with 60 nM semaglutide) were administered to the islets using syringe pumps at a speed of 0.25 mL/min for 30 min. Samples were collected at 1-min intervals, mixed, and stored at −20 °C. Insulin detection was performed using the Insulin Ultra-Sensitive low range HTRF kit (Cisbio, Codolet, France). Averages were calculated from three independent studies. Statistical differences between the two groups were evaluated by Student's *t*-test, using Graphpad Prism 8 software.

For the fluorescent imaging of insulin secretion, pancreatic islets were cultured on a glass-bottom confocal dish (D35-14-1-N; Cellvis, Mountain View, CA, USA) for 24 h, before they were washed in pre-warmed KRBB solution (125 mM NaCl, 5.9 mM KCl, 2.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1 mM L-Glutamine, 25 mM HEPES, 3 mM glucose, 0.01% BSA) for ~25 min to stop β-cell activity. Basal insulin secretion was recorded with a 10 μM Zn^{2+}-probe (PKZnR-5) for 5 min in 3 mM glucose KRBB solution, followed by stimulation of the islets with 11 mM glucose KRBB solution alone, or with GZR18 (60 nM), or semaglutide (60 nM). After stimulation of the islets, plasma membranes of the islet cells were labeled with 10 μM FM4-64 dye (T3166, Thermo Fisher, Waltham, MA, USA). All fluorescence images were acquired with a spinning-disc confocal microscope based on a CSU-X1 confocal scanner unit (Yokogawa, Musashino, Tokyo, Japan) and mounted on an inverted IX-81 Olympus microscope (Olympus Corporation, Shinjuku, Tokyo, Japan). Images were acquired by a 60x (NA1.35, Olympus Corporation) oil immersion objective lens, at a sampling rate of ~2Hz.

2.2. In vivo pharmacokinetics

2.2.1. Pharmacokinetic analysis of GZR18 injection in Sprague Dawley (SD) rats and cynomolgus monkeys

Pharmacokinetic parameters of GZR18 were analyzed in both SD rats and cynomolgus monkeys, which are commonly used species for pharmacokinetic assessment of this class of drugs. Before starting the study, the SD rats were kept in the laboratory for at least 3 days, and the cynomolgus monkeys at least 1 week to acclimatize, while their health was monitored by veterinarians.

The SD rats of approximately 220–320 g (n = 8 per group; four females and four males) and cynomolgus monkeys of approximately 2.5–4 kg (n = 6 per group; three females and three males) were treated with a single subcutaneous (s.c.) injection of GZR18 in multiple doses (SD rats 15, 90, 540 μg kg^{-1}; cynomolgus monkeys 10, 60, 360 μg kg^{-1}) to assess the pharmacokinetic characteristics of GZR18. A separate group was used to help calculate the bioavailability of GZR18 s.c. in SD rats and cynomolgus monkeys. SD rats (n = 8) were administered 90 μg kg^{-1} of GZR18 as an i.v. injection, and the cynomolgus monkeys (n = 6) received 60 μg mL^{-1} of i.v. GZR18.

Whole blood samples (~0.3 mL) were collected from the SD rats' retro-orbital sinus and cynomolgus monkeys using i.v. lines, in tubes containing dipotassium ethylenediaminetetraacetic acid (K_2-EDTA) anticoagulant, and centrifuged (2–8°C, 2000 g, 10 min) to collect plasma samples. The anesthetic used for this process was isoflurane. There were no major adverse events seen after blood sampling. For the SD rats, samples were taken for analysis at time points 0 (prior to administration) and 1, 3, 5, 8, 12, 16, 24, 36, 48, 72, 96, and 120 h following s.c. injection of GZR18; and at 0, 10, 30 min, and 1, 3, 5, 8, 12, 24, 48, 72, 96, and 120 h, following i.v. administration of GZR18. Cynomolgus monkey blood samples were collected for analysis at time points 0 (prior to administration) and 1, 3, 6, 8, 10, 12, 16, 24, 48, 72, 120, 168, and 240 h following s.c. administration of GZR18; and at 10 and 30 min following i.v. administration of GZR18.

In order to compare pharmacokinetic parameters of GZR18 with semaglutide, cynomolgus monkeys were administered with 60 μg kg^{-1} GZR18, or semaglutide, by either s.c. or i.v. (n = 4 per group). Whole blood samples (1 mL) were collected from the non-administration limb in K_2-EDTA anticoagulation tubes at different time points (0 and 10 min, and 1, 3, 6, 8, 10, 12, 16, 24, 48, 72, 120, 168, and 240 h). Samples were kept on ice for 2 h, followed by centrifugation at 2000*g* for 10 min at 2–8 °C.

Pharmacokinetic parameters of GZR18, including its terminal half-life ($T_{1/2}$), time to maximum concentration (T_{max}), maximum concentration (C_{max}), area under the curve (AUC), mean residence time, apparent volume of distribution, and clearance rate were determined by noncompartmental using Phoenix WinNonLin® version 6.4 (Certara USA, Princeton, NJ, USA).

2.2.2. Analysis of tissue distribution and excretion of 125I-GZR18 in SD rats

To investigate tissue distribution of GZR18 in SD rats, a total of 36 rats (18 males and 18 females; between 180–220 g) were sacrificed using carbon dioxide euthanasia at 4, 12, 48, and 96 h (n = 6 per time point), after s.c. administration of ^{125}I-GZR18 at a dose of 90 μg kg^{-1}. Before the start of the study, the SD rats were kept in the laboratory for at least 3 days, to acclimatize while their health was monitored by veterinarians. Femur, skeletal muscle, brain, intra-abdominal fat, gonad, stomach, duodenal intestine, colon mesenteric lymph nodes, adrenal glands, spleen, kidney, liver, thymus, heart, lung, and thyroid gland were analyzed. Bile samples were collected (n = 6) at intervals 0–4, 4–8, 8–12, 12–24, and 24–48 h to assess the distribution of ^{125}I-GZR18 in the bile. To assess the excretion of ^{125}I-GZR18, urine, and fecal samples were collected (n = 6) at intervals 0–1, 1–2, 2–4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168, hours after administration. If the cumulative excretion rate of feces and urine for 0–168 h was less than 90%, the time of sample collection was postponed a week until the cumulative excretion rate of feces and urine reached 90%, or until the excretion rate of feces and urine was less than 1% for 2 days. Radiation was measured using a γ counter (3470 WIZARD2, PerkinElmer, Waltham, MA, USA) and the radioactive concentration in the tissue and excretion samples was calculated.

2.3. In vivo efficacy

2.3.1. Single-dose of GZR18 in db/db mice

A total of 60 db/db mice (BKS.Cg-Dock7^{m}+/+Lepr$^{db/db}$; 8–9 weeks old), a widely used animal model of T2DM, with a nonfasting blood glucose of >20 mmol/L were divided into seven groups. Animals were fasted for 12—16 h prior to the start of the experiment. Animals were grouped according to body weight, nonfasting blood glucose, and fasting blood glucose. Group 1 consisted of the vehicle control group, and Group 2 to 7 was comprised of mice treated with 0.3, 1, 3, 10, 30, and 100 nmol kg^{-1} GZR18, respectively. GZR18 was administered as a single s.c. injection. Random blood glucose levels were measured before and after dosing at 1, 2, 3, 6, 12, 24, 48, and 72 h by a glucose meter (ACCU Check, Roche, Basel, Switzerland) through tail vein sampling. At the time of administration, 150 g food and 500 mL water were available; intake was monitored at 24, 48, and 72 h, after administration of GZR18 or the vehicle control. Body weight was also monitored before and after administration at 24, 48, and 72 h. GraphPad Prism (version 8.0) was used to create graphs and to calculate median effective dose (ED_{50}) values.

2.3.2. Repeated-dose of GZR18 in db/db mice

In total, 60 db/db mice (BKS.Cg-Dock7^{m}+/+Lepr$^{db/db}$; 8–15 weeks old; 30 males and 30 females) were divided into five groups according to six parameters (nonfasting blood glucose, fasting blood glucose, triglyceride [TG], total cholesterol [TC], HbA_{1c}, and body weight). The db/db groups included a model control group, a dulaglutide control group (300 μg kg^{-1}), and low- (30 μg kg^{-1}), mid- (100 μg kg^{-1}), and high-dose (300 μg kg^{-1}) GZR18 groups. In addition, 14 male mice were used as healthy controls. Vehicle or GZR18 was administrated by s.c. administration once every three days for 33 days, i.e. 11 doses. For the assessment of nonfasting blood glucose, blood samples were collected from the tail tip before each administration of vehicle, dulaglutide, or GZR18 and

at 3, 6, 9, 12, 24, 48, and 72 h after the first and last administration. Samples were analyzed using a blood glucose meter (NOVA Biomedical Corporation, Waltham, MA, USA). The animals in each group were fasted for 6 h prior to administration of the first dose but had free access to water. Fasting blood glucose levels were analyzed using ACCI-CHEK Performa (Roche, Basel, Switzerland) 48 h after the third, fifth, and 11th administration of vehicle, dulaglutide, or GZR18. To measure HbA_{1c} levels, blood samples were collected from the tail tip before dividing the mice into groups 24 h after the 6th and 11th administration. The level of HbA_{1c} was determined with biochemical analysis equipment (HITACHI7180, Beijing Leadman Biochemistry Co., Ltd, Beijing, China) according to the manufacturer's instructions.

Intraperitoneal glucose tolerance tests (ipGTTs) were performed 48 h after the first and 10th injection with vehicle, dulaglutide, or GZR18. Blood glucose was measured at 30, 60, 120, and 180 min after glucose loading (0.5 g kg^{-1}, 0.05 mL 10 g^{-1}), and the area under the blood glucose–time curve was calculated. In addition, blood samples were collected 10 and 30 min after glucose administration for the ipGTT, for the assessment of fasting serum levels. Serum samples were obtained before administration, and at the end of the experiment, to detect the main blood lipids, fasting TG and TC levels. Food, water intake and body weight were monitored at the beginning of the study and before each drug administration. The average daily food intake, water intake, and weight changes of each mouse, and that of each group, were calculated.

At the end of the experiment, mice were euthanized using an overdose of isoflurane, and death was confirmed by femoral artery bloodletting. The mice required for blood collection were euthanized by bloodletting after the procedure of blood collection. Statistical analysis was performed using SPSS software (PASW Statistics 18.0.0) to run analysis of variance (ANOVA). If one-way ANOVA showed statistical significance ($p < 0.05$) and the variance was homogeneous, Dunnett's multiple comparisons test was used for group comparison analyses; if the variance was not homogeneous, Dunnett's T3 test was used for group comparison analyses.

2.4. Data and statistical analysis

Statistical significance (for the purpose of this study) was defined as $p < 0.05$. The effects of a single dose or repeated doses of GZR18, semaglutide, or dulaglitide *in vitro* or *in vivo,* were assessed using one-way ANOVA analysis. Statistical analysis was carried out using GraphPad Prism or PASW Statistics as indicated in the respective Method sections. The sample sizes used for specific experiments are also mentioned in the respective Method sections. Pharmacokinetic parameters were determined by non-compartmental methods, using Phoenix WinNonLin v6.4 (Pharsight Inc.).

2.5. Animal welfare

All experimental procedures followed methods that were approved by local animal welfare committees (The effect of GZR18 on GSIS, Animal Experimental Ethics Committee of Gan & Lee Pharmaceuticals [IACUC] No. IACUC-2020-167; pharmacokinetic analysis in SD rats and cynomolgus monkeys, Institutional Animal Care and Use Committee [IACUC] No. IACUC2020011 and W00192, United-Power Pharma Tech Co., LTD [UPP]; tissue distribution and excretion analysis in SD rats, IACUC No. UPP-IACUC-2020-10001, UPP [Beijing] Pharmaceutical Research Co., Ltd; single-dose analysis of GZR18 in db/db mice, IACUC No. IACUC-2020-080, Gan & Lee Pharmaceuticals; repeated-dose analysis of GZR18 in db/db mice, IACUC No. UPP-IACUC-2020-G5802PDMo1, UPP [Beijing] Pharmaceutical Research Co., Ltd). Where possible, any pain, tension, or discomfort caused to the animals was either avoided or minimized during the experiments. In the case in which the animals were to experience short-term mild pain or discomfort during the experiment, analgesics or anesthetics were given to relieve the symptoms. Permission from the animal ethics committee was required if the aforementioned actions could not be followed for any procedures deemed necessary by the project leader or the veterinarian.

Table 1
The binding affinity of GZR18, liraglutide, semaglutide, and GLP-1 (7–37) to GLP-1 receptor.

Sample	IC_{50} (nM) mean ± SD	
	0.005% HSA	2% HSA
GZR18	3.67 ± 1.95	5552.33 ± 378.83
Liraglutide	0.52 ± 0.34	29.44 ± 13.01
Semaglutide	0.75 ± 0.29	609.57 ± 51.33
GLP-1 (7–37)	2.82 ± 1.07	1.50 ± 0.24

GLP-1, glucagon-like peptide-1; HSA, human serum albumin; IC_{50}, half maximal inhibitory concentration; SD, standard deviation; n =3; 1 nmol of GZR18 is 4.139 μg.

Table 2
The binding affinity of GZR18, liraglutide, semaglutide, and GLP-1 (7–37) to HSA.

Sample	EC_{50} (nM) mean ± SD		Ratio EC_{50}
	0.005% HSA	2% HSA	
GZR18	1.91 ± 0.51	696.0 ± 149.80	365
Semaglutide	0.21 ± 0.06	100.0 ± 27.00	478
Liraglutide	0.28 ± 0.04	10.61 ± 4.80	38
GLP-1 (7–37)	0.18 ± 0.01	1.148 ± 0.01	6

EC_{50}, half maximal effective concentration; GLP-1, glucagon-like peptide-1; HSA, human serum albumin; SD, standard deviation; n = 3; 1 nmol of GZR18 is 4.139 μg.

3. Results

3.1. In vitro pharmacology and activity

3.1.1. GZR18 has a lower receptor binding affinity in the presence of HSA

Results indicated that the receptor binding affinity of GZR18 was similar to GLP-1 (7–37), but weaker than liraglutide and semaglutide under low concentrations of HSA. In contrast to GLP-1 (7–37), the receptor binding affinities of GZR18, liraglutide, and semaglutide, were reduced by higher concentrations of HSA, with the binding affinity of GZR18 decreasing the most, suggesting that its binding affinity to HSA was stronger than that of liraglutide and semaglutide. When HSA levels were increased to 2%, the IC_{50} of GZR18, liraglutide, and semaglutide, measured 3701.55-, 19.63-, and 406.38-fold higher, respectively, when compared with GLP-1 (7–37) (Table 1).

3.1.2. GZR18 displays a higher affinity with HSA

GZR18 showed higher biological activity than liraglutide or GLP-1 (7–37), indicating higher EC_{50} ratios. EC_{50} ratios were calculated to determine GZR18 affinity for HSA, a surrogate of biological activity. An increase in HSA levels from 0.005 to 2% resulted in a rise in biological activity across molecules (Table 2). The EC_{50} ratios were highest for GZR18 (365) and semaglutide (478) and notably lower for liraglutide (38) and GLP-1 (7–37) (6).

3.1.3. GZR18 dose-dependently stimulates insulin secretion in mouse islets

GZR18 promoted glucose-dependent insulin secretion under high glucose concentrations in primary mouse pancreatic islet β-cells to a similar extent as semaglutide, which was consistent with the results of islet perifusion and whole islets fluorescence imaging experiments.

Incubation with GZR18 concentrations of 30 and 90 nM increased glucose-dependent insulin secretion 1.7- and 3.5-fold ($p < 0.05$, $p < 0.001$), respectively, in a dose-dependent manner, compared with the high-glucose stimulation group. Under the same test conditions, the semaglutide control group significantly promoted insulin release ($p <$

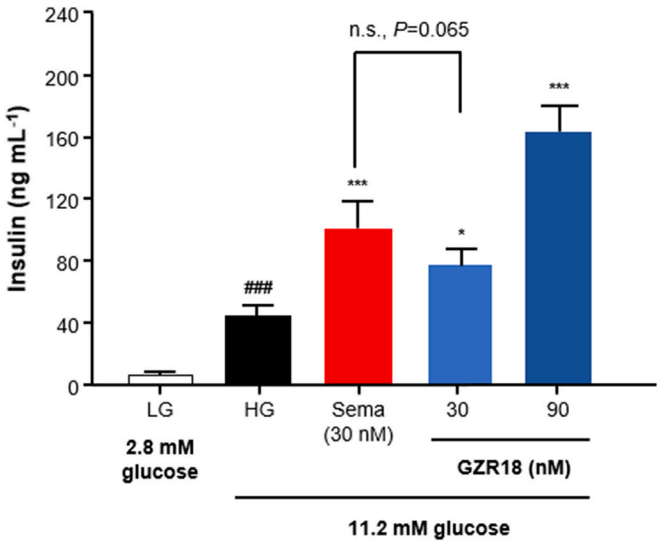


Fig. 1. The effect of a single dose of semaglutide and GZR18 on GSIS *in vitro*. 1 nmol of GZR18 is 4.139 μg.
$^{*}p < 0.05$, $^{***}p < 0.001$ versus high-glucose stimulation; $^{\#\#\#}p < 0.001$ high-glucose stimulation versus low-glucose stimulation
GSIS, glucose-stimulated insulin secretion; HG, high-glucose stimulation; LG, low-glucose stimulation; n.s., not significant; Sema, semaglutide.

0.001) by two-fold compared with the high-glucose stimulation group. Moreover, there was no significant difference between the 30 nM semaglutide control group and the same dose of GZR18 ($p > 0.05$) (Fig. 1).

Islet perifusion showed that in comparison with the 11.1 mM glucose stimulation group (control), GZR18 (60 nM) or semaglutide (60 nM) significantly stimulated insulin secretion of pancreatic β-cells under high-glucose (11.1 mM) ($p < 0.05$). The total amount of insulin secretion $(AUC)_{10–40min}$ was 1.9 times higher than that of the control group. No significant differences in AUC were measured between GZR18 and semaglutide (Fig. 2A–D). Fluorescence imaging of the whole pancreatic islets showed the same intensity of glucose-dependent promotion of insulin secretion with GZR18 (60 nM) or semaglutide (60 nM) compared to the control group (Fig. 2E). Real time fluorescence imaging revealed that GZR18 promotes insuline secretion by recruitment of responsive β-cells and by enhancing the secretory capacity of the β-cells.

3.2. *In vivo pharmacokinetics*

3.2.1. *GZR18 may be suitable for a once-weekly dosing schedule*

There were no significant differences in $T_{1/2}$ and clearance rate among the different GZR18 dose groups. The results demonstrated that administration of GZR18 resulted in linear pharmacokinetic characteristics in SD rats within the dose range of 15–540 μg kg^{-1} (Fig. 3A, Table 3). The absolute bioavailability of s.c. GZR18 in SD rats was measured as 41.8% following administration of 90 μg kg^{-1} GZR18.

Similar to the results observed in SD rats, pharmacokinetic analysis in cynomolgus monkeys revealed increased exposure levels of GZR18 in line with dose increases (Fig. 3B). No significant differences in $T_{1/2}$ and clearance rate were observed among the different GZR18 dose groups. Therefore, the results indicated that GZR18 exhibited linear pharmacokinetic characteristics in cynomolgus monkeys within the dose range of 10–360 μg kg^{-1} (Supplementary Table 1). The absolute bioavailability of s.c. GZR18 in cynomolgus monkeys was measured as 57.0%.

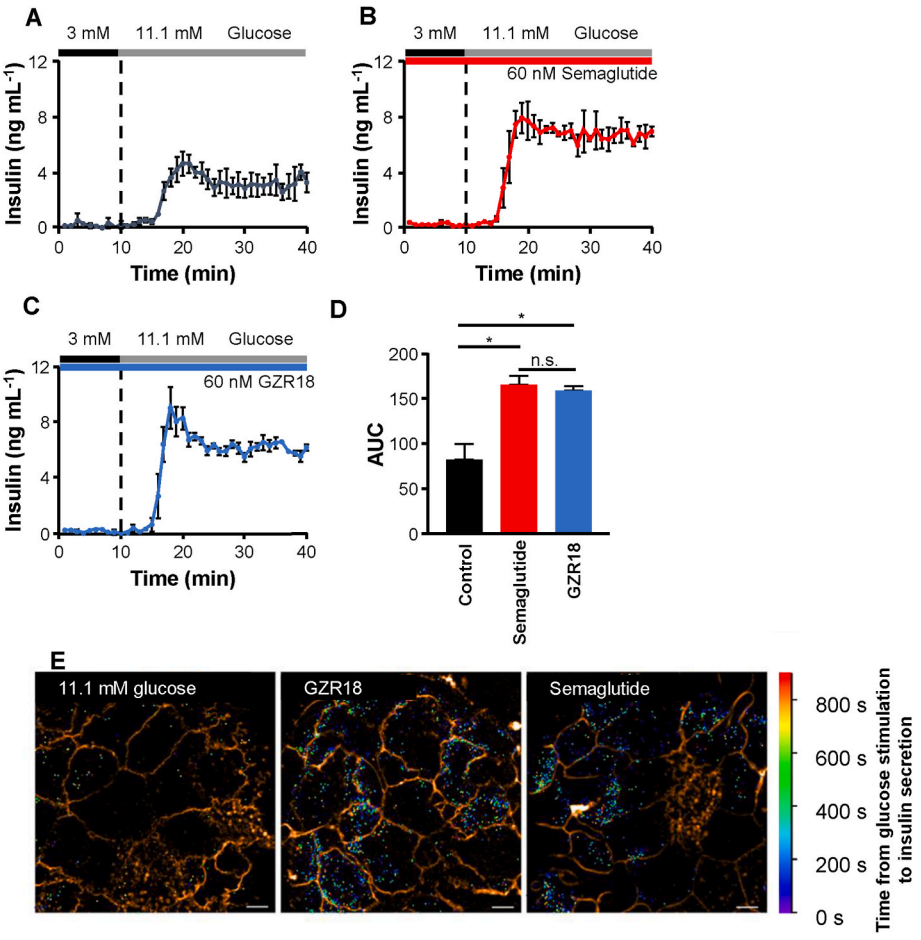


Fig. 2. The effect of GZR18 and semaglutide on glucose-stimulated insulin secretion. Dynamic insulin secretion stimulated with 11.1 mM glucose alone (A), 11.1 mM glucose with 60 nM semaglutide (B), or 11.1 mM glucose with 60 nM GZR18 (C). (D) The total amount of insulin secretion (AUC) under high glucose stimulation (10–40 min). (E) Imaging insulin secretion of β-cells stimulated with high-glucose, high-glucose with 60 nM GZR18, or high-glucose with 60 nM semaglutide. Insulin granule fused sites are coloured to indicate time from glucose stimulation to insulin release. The plasma membrane of islet cells is labelled by FM4-64 (orange). Scale bar = 10 μm.
1 nmol of GZR18 is 4.139 μg.
$^{*}p < 0.05$ vs high-glucose stimulation; n.s., not significant; AUC, area under the curve.

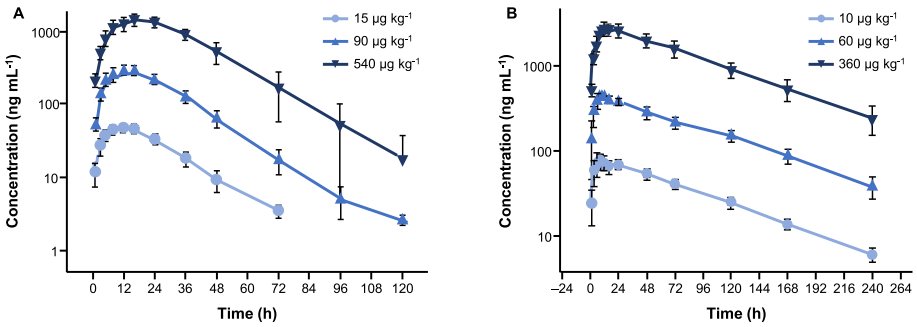


Fig. 3. Plasma concentrations of GZR18 following a single subcutaneous injection in Sprague Dawley rats and cynomolgus monkeys. (A). The mean plasma concentration plotted against time following a single subcutaneous administration of GZR18 at concentrations of 15, 90, and 540 μg kg^{-1} in Sprague Dawley rats. (B). The mean plasma concentration plotted against time following a single subcutaneous administration of GZR18 at concentrations of 10, 60, and 360 μg kg^{-1} in cynomolgus monkeys.
1 nmol of GZR18 is 4.139 μg.

Table 3
Mean pharmacokinetic parameters following administration of GZR18 in Sprague Dawley rats.

PK parameters	s.c.			i.v.
	15 μg kg^{-1}	90 μg kg^{-1}	540 μg kg^{-1}	90 μg kg^{-1}
$T_{1/2}$ (h)	12.5 ± 1.32	13.0 ± 1.58	13.3 ± 2.35	13.0 ± 2.46
T_{max} (h)	12.5 ± 2.56	13.5 ± 2.98	18.0 ± 5.24	0.04 ± 0.05
C_{max} (ng mL^{-1})	48.6 ± 5.83	296 ± 25.6	1490 ± 250	2540 ± 462
$AUC_{(0-t)}$ (h ng^{-1} mL^{-1})	1490 ± 229	9950 ± 996	61,500 ± 10,400	23,800 ± 4100
$AUC_{(0-72\ h)}$ (h ng^{-1} mL^{-1})	1530 ± 202	9650 ± 905	57,900 ± 8490	NA
$AUC_{(0-inf)}$ (h ng^{-1} mL^{-1})	1580 ± 220	10,000 ± 1010	61,900 ± 10,900	23,800 ± 4110
$AUC_{\_\%Extrap}$ (%)	5.87 ± 2.15	0.72 ± 0.40	0.58 ± 0.64	0.34 ± 0.17
MRT (h)	21.2 ± 3.46	25.2 ± 2.72	30.4 ± 4.33	12.5 ± 1.10
V_d/F (kg^{-1} mL^{-1})	173 ± 18.9	168 ± 16.8	170 ± 29.0	49.4 ± 6.73
CL/F (h g^{-1} mL^{-1})	9.63 ± 1.12	9.07 ± 1.03	8.94 ± 1.44	3.88 ± 0.73
F (%)	NA	41.8	NA	NA

AUC, area under the curve; CL, clearance; C_{max}, maximum concentration; Extrap, extrapolation; F, absolute bioavailability; inf, infinity; i.v., intravenous; MRT, mean residence time; NA, not applicable; PK, pharmacokinetics; s.c., subcutaneous; SD standard deviation; $T_{1/2}$, terminal half-life; T_{max}, time to maximum concentration; V_d, apparent volume of distribution; Mean ± SD; 1 nmol of GZR18 is 4.139 μg.

The comparative pharmacokinetics study of GZR18 versus semaglutide demonstrated similar plasma concentration–time change characteristics and bioavailability after s.c or i.v. administration. A 60 μg kg^{-1} s.c. dose of GZR18 or semaglutide in cynomolgus monkeys showed an average $T_{1/2}$ of 61.3 and 58.1 h, respectively, and a T_{max} of 14 versus 12 h, respectively. The C_{max} of GZR18 (527 ± 140 ng mL^{-1}) was lower than that of semaglutide (700 ± 127 ng mL^{-1}). In addition, the bioavailability was 73.3% for s.c. GZR18 and 70.5% for s.c. semaglutide in cynomolgus monkeys (Table 4).

3.2.2. GZR18 is mainly distributed in serum

GZR18 was mainly distributed in the serum ($AUC_{(0-96\ h)}$ = 14,482.5 ng Equ/g[mL]*h) and in tissues and organs with high excretion and blood flow (Supplementary Fig. 1A). The organ with the least amount of total radioactivity concentration of GZR18 was the brain ($AUC_{(0-96\ h)}$ = 465.4 ng Equ/g[mL]*h). The cumulative distribution of radioactivity in the bile within 48 h was 4.55 ± 1.17% of the total injected ^{125}I-GZR18 in SD rats (Supplementary Fig. 1B). The cumulative excretion of ^{125}I-GZR18 in feces and urine at 648 h reached 12.33 ± 3.46% and 58.30 ± 3.9%, respectively, indicating that GZR18 was mainly excreted through urine (Supplementary Fig. 1C).

Table 4
Mean pharmacokinetic parameters following administration of GZR18 and semaglutide in cynomolgus monkeys.

PK parameters	GZR18		Semaglutide	
	s.c. 60 μg kg^{-1}	i.v. 60 μg kg^{-1}	s.c. 60 μg kg^{-1}	i.v. 60 μg kg^{-1}
$T_{1/2}$ (h)	61.3 ± 5.34	61.6 ± 7.32	58.1 ± 1.57	62.8 ± 4.68
T_{max} (h)	14 ± 6.73	0.167 ± 0.00	12.0 ± 2.83	0.167 ± 0.00
C_{max} (ng mL^{-1})	527 ± 140	1640 ± 0.00	700 ± 127	2140 ± 203
$AUC_{(0-t)}$ (h ng^{-1} mL^{-1})	51,800 ± 9700	70,700 ± 3730	62,100 ± 7940	88,100 ± 4390
$AUC_{(0-inf)}$ (h ng^{-1} mL^{-1})	55,700 ± 10900	74,800 ± 2780	65,900 ± 8550	93,600 ± 4170
$AUC_{\_\%Extrap}$ (%)	7.05 ± 0.954	5.41 ± 1.48	5.80 ± 0.755	5.91 ± 1.27
MRT (h)	75.2 ± 1.75	59.2 ± 1.81	71.1 ± 3.32	61.5 ± 3.31
V_d/F (kg^{-1} mL^{-1})	96.9 ± 13.3	59.4 ± 7.25	77.2 ± 9.24	49.7 ± 5.72
CL/F (h g^{-1} mL^{-1})	1.10 ± 0.188	0.803 ± 0.0298	0.924 ± 0.136	0.642 ± 0.0289
K_{el} (h^{-1})	0.0114 ± 0.000956	0.0113 ± 0.00135	0.0119 ± 0.000329	0.0111 ± 0.000845
F (%)	73.3	NA	70.5	NA

AUC, area under the curve; CL, clearance; C_{max}, maximum concentration; Extrap, extrapolation; F, absolute bioavailability; inf, infinity; i.v., intravenous; MRT, mean residence time; NA, not applicable; PK, pharmacokinetics; s.c., subcutaneous; SD standard deviation; $T_{1/2}$, terminal half-life; T_{max}, time to maximum concentration; V_d, apparent volume of distribution; Mean ± SD; 1 nmol of GZR18 is 4.139 μg.

3.3. In vivo efficacy

3.3.1. Single-dose GZR18 lowers blood glucose levels

A single dose of GZR18 in db/db mice reduced nonfasting blood glucose, food and water intake, and body weight in a dose-dependent manner. Changes in nonfasting blood glucose levels were observed following the administration of a single dose of GZR18 in db/db mice (Fig. 4). Nonfasting blood glucose levels in the 3, 10, 30, and 100 nmol kg^{-1} GZR18 groups were significantly reduced compared with the vehicle control group at the three time points (6, 12, and 24 h) (Fig. 4A, C and E). The ED_{50} values of GZR18 were 3.02 nmol kg^{-1} (Figs. 4B), 8.45 nmol kg^{-1} (Fig. 4D), and 10.27 nmol kg^{-1} (Fig. 4F) at 6, 12, and 24 h, respectively. The blood-glucose-lowering effect of 10–300 nmol kg^{-1} GZR18 was maintained for 72 h (Fig. 4G and H). Food and water intake of mice in the 1, 3, 10, 30, and 100 nmol kg^{-1} GZR18 treatment groups were significantly reduced compared with the vehicle control group 24 h after treatment ($p < 0.05$). Significant differences persisted for 48 h after treatment in the 10, 30, and 100 nmol kg^{-1} GZR18 groups ($p < 0.01$) and for 72 h after treatment in the 30 or 100 nmol kg^{-1} GZR18 groups ($p < 0.01$) (Fig. 5A and B). Compared with the vehicle control group, the body weight of mice in the 3, 10, 30, and 100 nmol kg^{-1} GZR18 groups

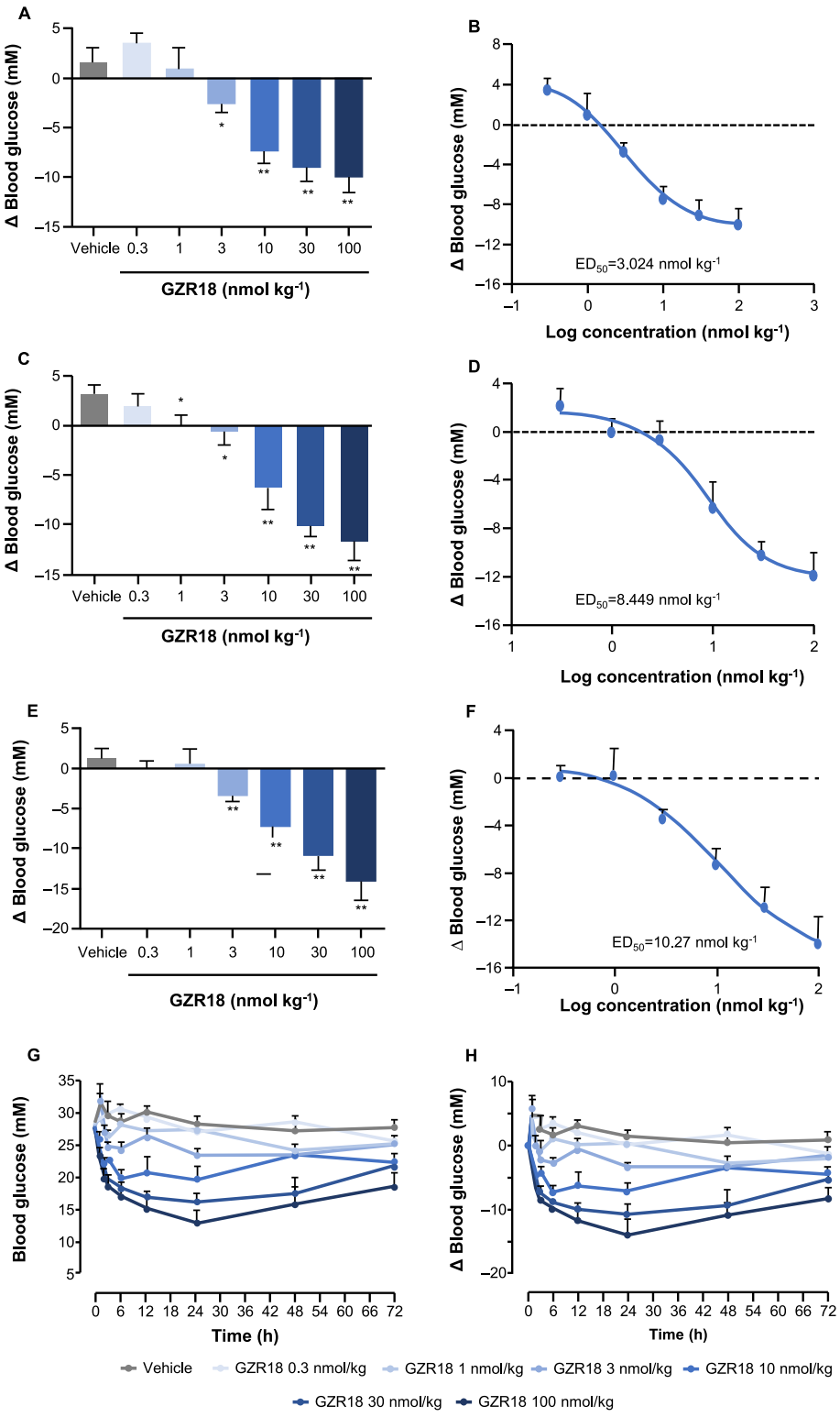


Fig. 4. The effect of a single dose of GZR18 on nonfasting blood glucose in db/db mice. Changes in nonfasting blood glucose levels and concentration curves in db/db mice at 6 h (A, B), 12 h (C, D) and 24 h (E, F) after administration of GZR18. $^{*}p < 0.05$, $^{**}p < 0.01$ versus model control. Nonfasting blood glucose levels (G) and changes in blood glucose levels (H) in db/db mice after administration of GZR18.
1 nmol of GZR18 is 4.139 μg.
ED_{50}, median effective dose.

decreased significantly after 24 ($p < 0.01$) and 48 h ($p < 0.05$). Moreover, after 72 h of treatment, the body weight of the mice in the 10, 30, and 100 nmol kg^{-1} GZR18 groups remained significantly reduced ($p < 0.01$) (Fig. 5C).

3.3.2. *Repeat doses of GZR18 improve glucose homeostasis*

GZR18 lowered nonfasting blood glucose and fasting blood glucose in db/db mice in a dose-dependent manner compared with the model control group. Nonfasting blood glucose levels in db/db mice were significantly lowered across 30–300 μg kg^{-1} GZR18 dosing groups after the first and 11th consecutive treatment. The area under the curve with respect to glucose (G-AUC) on day 3 and day 36 was reduced in all GZR18 groups ($p < 0.05$) and the dulaglutide control group ($p < 0.001$), compared with the model control group (Supplementary Figs. 2A and B). The mid-, and high-dose GZR18 reduced fasting blood glucose levels in db/db mice at 48 h after the third dose compared with the model control

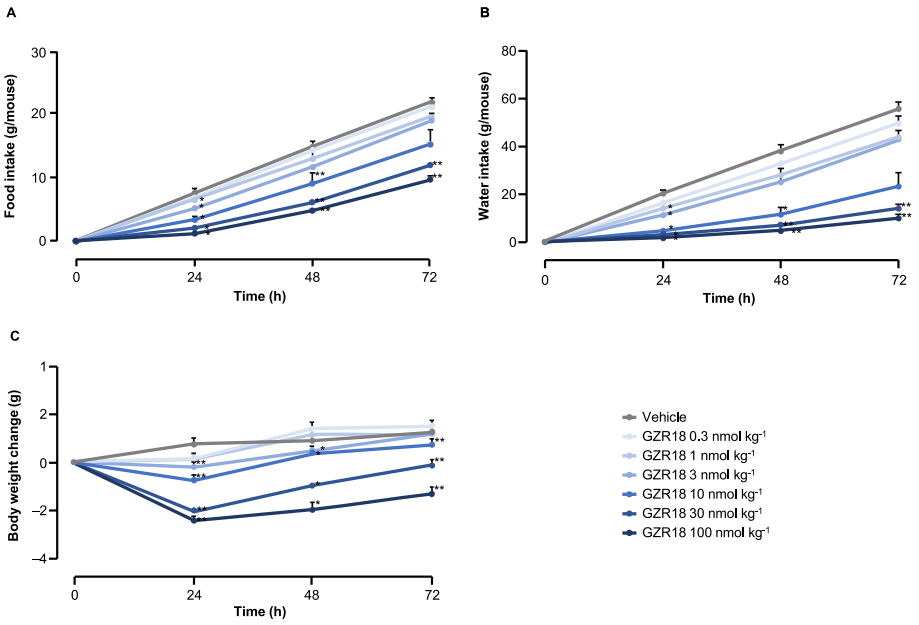


Fig. 5. The effect of a single dose of GZR18 on food intake, water intake, and body weight of db/db mice. (A). Changes in food intake in db/db mice treated with GZR18. (B). Changes in water intake in db/db mice treated with GZR18. (C). Changes in body weight of db/db mice treated with GZR18. $^{*}p < 0.05$, $^{**}p < 0.01$ versus vehicle.
1 nmol of GZR18 is 4.139 μg.

group ($p < 0.05$). The fasting blood glucose levels of mice in each group treated with GZR18 significantly decreased after administration of the fifth and 11th dose ($p < 0.01$) (Fig. 6A). Mice administered with GZR18 had lower HbA_{1c} levels compared with the model control group. HbA_{1c} measurements after the sixth and 11th dose showed that GZR18 significantly reduced HbA_{1c} levels in a dose-dependent manner compared with the model control group. Dulaglutide (300 μg kg^{-1}) also significantly lowered HbA_{1c} levels after both the sixth and 11th treatment compared with the model control group ($p < 0.01$) (Fig. 6B). The glucose tolerance of diabetic mice was improved after administration of GZR18 compared with the model control group. After the first dose, the G-AUC of the ipGTT was significantly increased in the 100 and 300 μg kg^{-1} GZR18 dose groups ($p < 0.001$ versus model control group) and for the dulaglutide control group ($p < 0.01$ versus model control group) (Fig. 6C). At the 10th dose, the G-AUC of the ipGTT was significantly reduced for the 100 μg kg^{-1} GZR18 dose group ($p < 0.01$ versus model control group) and the 300 μg kg^{-1} GZR18 dose group ($p < 0.001$ versus model control group) (Fig. 6D). At 48 h after the 1st dose, both GZR18 and dulaglutide showed a trend towards increased amounts of secreted first-phase insulin compared with that of the model control mice but no significant difference was observed, owing to the large variation among individuals (Supplementary Fig. 2C). At 48 h after the 10th dose there was a significant increase in secreted insulin in the 100 and 300 μg kg^{-1} GZR18 dose groups at time points 0 and 10 min compared with that of the model control mice (Fig. 6E).

GZR18 lowered serum TG, but not serum TC, levels in diabetic mice. Compared with the model control group, the GZR18 low-dose, mid-dose, and high-dose group, and the dulaglutide control group, decreased TG levels by 26.1%, 34.8%, 54.9%, and 36.5%, respectively. The high-dose GZR18 group showed significantly reduced serum TG levels ($p < 0.001$) (Supplementary Fig. 2D). Neither GZR18 nor dulaglutide significantly affected serum TC levels compared with the model control group (data not shown).

Compared with that of the model control group, the cumulative food and water intake of mice in the GZR18 groups were significantly decreased ($p < 0.001$) following treatment. Compared to the model control group, mid-dose GZR18 treatment resulted in decreased food intake similar to dulaglutide, while for water intake, the low-dose GZR18 group displayed reductions similar to the dulaglutide control group (Fig. 6F and G). There were no significant changes in body weight for the GZR18 dose groups and dulaglutide compared to the model control group (Fig. 6H).

4. Discussion

GZR18 is an investigational long-lasting GLP-1 analog developed for the management of T2DM. This study investigated the pharmacokinetics and preclinical effects of GZR18 in GLP-1 receptor-expressing cell lines, primary mice islets, and animal models (db/db mice, SD rats, cynomolgus monkeys).

GLP-1 analogs of the native human GLP-1 provide more significant pharmacological effects than endogenous GLP-1 (Andersen et al., 2018; Cornell, 2020). GLP-1 receptor agonists can bind plasma proteins, such as albumin, to reduce free drug concentrations in the plasma, thereby attenuating drug potency *in vivo* (Trainor, 2007). Indeed, *in vitro* binding results showed that in contrast to endogenous GLP-1 (7–37), the GLP-1 receptor-binding affinity of GZR18, liraglutide, and semaglutide was notably reduced by high concentrations of HSA, with the binding affinity of liraglutide being reduced the least, suggesting that the binding affinities of GZR18 and semaglutide to HSA were more robust than that of liraglutide.

It is also hypothesized that an increased albumin affinity can extend the half-life of GLP-1 receptor agonists through decreased renal clearance and protection from metabolic degradation. Both semaglutide and GZR18 were found to have increased albumin affinity in this study; their half-lives in cynomolgus monkeys were observed to be 58.1 h and 61.3 h, respectively, which suggests that GZR18 may be suitable for a similar once-weekly dosing schedule as is currently approved for semaglutide (Novo Nordisk A/S, 2017). One of the main advantages of long-acting GLP-1 receptor agonists over short-acting GLP-1 receptor agonists is the reduced injection burden (Giorgino et al., 2018). This would benefit patients who are unable to adhere to frequently administered therapy, who are unwilling to self-inject, or are dependent on caregivers for injection. Poor glycemic control in patients with diabetes can be a consequence of suboptimal adherence to antihyperglycemic medications, which may also lead to increased hospitalization, diabetic complications, and increased use of healthcare resources. More convenient dosing schedules, such as once-weekly GLP-1 receptor agonists, may lead to improved adherence to such medication (Giorgino et al., 2018).

The main purpose of prescribing GLP-1 receptor agonists to patients with T2DM is to obtain good glycemic control by stimulating insulin release from pancreatic cells in a glucose-dependent manner and by

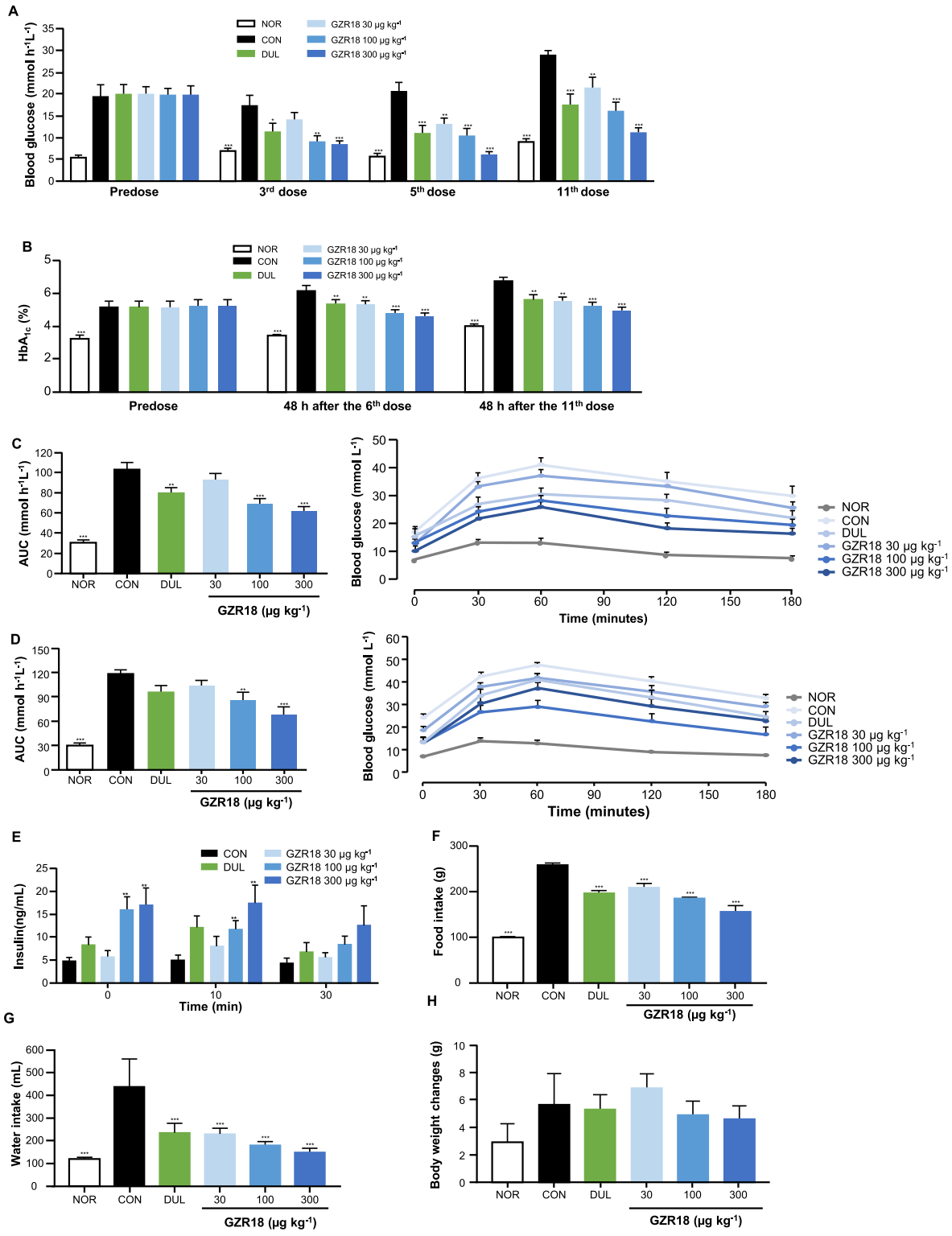


Fig. 6. The effect of repeated doses of GZR18 on glucose and lipid metabolism in db/db mice. (A) The effect of successive administration of GZR18 on fasting blood glucose in db/db mice. (B) HbA_{1c} levels of db/db mice before administration, and 48 h after the sixth and 11th administration of GZR18. (C) The effect of the first dose of GZR18 on ipGTT in db/db mice after the first dose. (D) The effect of the 10th dose of GZR18 on ipGTT in db/db mice. (E) Fasting blood insulin levels at 48 h after 10 successive doses of GZR18 and blood insulin levels after glucose stimulation. The effects of repeated administration of GZR18 on food intake (F), water intake (G), and body weight (H). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus CON.

AUC, area under the curve; CON, model control group; DUL, dulaglutide control group; G-AUC, glucose area under the curve; HbA_{1c}, glycated hemoglobin; ipGTT, intraperitoneal glucose tolerance test; NOR, healthy control group.

suppressing glucagon secretion (Cornell, 2020). The degree of the achieved glycemic control appears to depend on the half-lives and the mode of action of the GLP-1 receptor agonists (Cornell, 2020). GZR18 reduced nonfasting blood glucose levels and maintained this lowering effect for up to 72 h in db/db mice injected with a single dose. Continued administration of GZR18 decreased both nonfasting and fasting glucose levels, and HbA_{1c} levels were significantly reduced in a dose-dependent manner. These results indicate that GLP-1 receptor agonist GZR18 could provide clinically meaningful improvement of glycemic control.

Multiple phase 3 trials have demonstrated that GLP-1 receptor agonists also reduce food intake and promote body weight loss, with the extent of weight loss varying between compounds (Andersen et al., 2018). The US Food and Drug Administration (FDA) and the European Medicines Agency approved once-daily liraglutide for the treatment of obesity (Andersen et al., 2018; Novo Nordisk A/S, 2015; Novo Nordisk A/S, 2018), and recently, once-weekly semaglutide received approval from the FDA for chronic weight management in adults with obesity. In line with the known effects of GLP-1 receptor agonists on weight loss, GZR18 induced body weight loss in mice following single dose administration, indicating a potential role for GZR18 in the weight management of obese patients with and without T2DM. However, prolonged administration did not reduce body weight.

The most commonly reported adverse effects for once-weekly GLP-1 receptor analogs are gastrointestinal disorders. Nausea has been reported as the most common adverse effect, and its episodes seem to be dose-dependent and diminish with ongoing treatment (Filippatos et al., 2014; O'Neil et al., 2018). The bioavailability (amount of active drug that enters the circulation when administered into the body) of s.c. GZR18 was similar to s.c. semaglutide in the pharmacokinetics comparison study in cynomolgus monkeys. However, a difference in C_{max} between GZR18 and the once-weekly GLP-1 analog, semaglutide, was observed; which may be due to GZR18 having a longer fatty-acid side chain than semaglutide, thus resulting in a higher binding affinity for serum albumin, and may lead to a difference in adverse events.

This was a preclinical investigation of GZR18 using animal models and caution should be taken when extrapolating these results to potential clinical benefits in human subjects. One limitation of the *in vivo* studies was that the analyses of the GZR18 study groups compared GZR18 against vehicle controls, not against active control groups. This limits the conclusions that can be drawn about the performance of GZR18 in relation to currently available GLP-1 receptor agonists. Moreover, the dosing schedule in the efficacy study using repeated doses of GZR18 was set to every 3 days, and therefore conclusions about optimal dosing could not be made.

The studies described in this manuscript demonstrated that GZR18 could specifically bind and activate GLP-1 receptor and effectively control glucose levels in db/db mice by increasing insulin secretion. Thus, GZR18 may have potential to treat diabetic patients as a once-weekly, long-acting GLP-1 analog. Based on our research, clinical studies of GZR18 are warranted.

Funding

The study was supported by Gan & Lee Pharmaceuticals. The authors that are employees of Gan & Lee Pharmaceuticals were involved in the design of the study, collection, analysis, and interpretation of data, and in writing the manuscript.

CRediT author statement

MZ, YZ, XP, AH, YW, YD, CC, FX, BW, WX, YQ, and WC contributed to data analysis, study conduction, and editing of the manuscript. MM contributed to the project management of the study. WC contributed to the conceptualization of the study, interpretation of the study data and editing of the manuscript. All authors have read and approved the final manuscript.

Declaration of competing interest

All authors, except Xiaohong Peng, are employees of Gan & Lee Pharmaceuticals.

Xiaohong Peng: None.

Acknowledgements

The study was funded by Gan & Lee Pharmaceuticals. The authors acknowledge Naveen Samuel of Gan & Lee Pharmaceuticals for his support with the preparation of the manuscript, Dr Zhongru Gan for his contributions, and Dr Viola Kooij, PhD, of integrated medhealth communication (imc), UK, for medical writing support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejphar.2022.175107.

References

Andersen, A., Lund, A., Knop, F.K., Vilsboll, T., 2018. Glucagon-like peptide 1 in health and disease. Nat. Rev. Endocrinol. 14 (7), 390–403. https://doi.org/10.1038/s41574-018-0016-2.

Chun, J.H., Butts, A., 2020. Long-acting GLP-1RAs: an overview of efficacy, safety, and their role in type 2 diabetes management. JAAPA 33 (S8 Suppl. 1), 3–18. https://doi.org/10.1097/01.JAA.0000669456.13763.bd.

Cornell, S., 2020. A review of GLP-1 receptor agonists in type 2 diabetes: a focus on the mechanism of action of once-weekly agents. J. Clin. Pharm. Therapeut. 45 (Suppl. 1), 17–27. https://doi.org/10.1111/jcpt.13230.

Filippatos, T.D., Panagiotopoulou, T.V., Elisaf, M.S., 2014. Adverse effects of GLP-1 receptor agonists. Rev. Diabet. Stud. 11 (3–4), 202–230. https://doi.org/10.1900/rds.2014.11.202.

Gan, Z., Wei, C., Fangkai, X., Lingyu, C., Jianghong, N., Bin, M., 2021. Long-acting GLP-1 compound. Gan Lee Pharmaceut. WO2021/136303, 2021–2027, 8.

Giorgino, F., Penfornis, A., Pechtner, V., Gentilella, R., Corcos, A., 2018. Adherence to antihyperglycemic medications and glucagon-like peptide 1-receptor agonists in type 2 diabetes: clinical consequences and strategies for improvement. Patient Prefer. Adherence 12, 707–719. https://doi.org/10.2147/ppa.S151736.

Lau, J., Bloch, P., Schaffer, L., Pettersson, I., Spetzler, J., Kofoed, J., Kruse, T., 2015. Discovery of the once-weekly glucagon-like peptide-1 (GLP-1) analogue semaglutide. J. Med. Chem. 58 (18), 7370–7380. https://doi.org/10.1021/acs.jmedchem.5b00726.

Nauck, M.A., Meier, J.J., 2016. The incretin effect in healthy individuals and those with type 2 diabetes: physiology, pathophysiology, and response to therapeutic interventions. Lancet Diabetes Endocrinol. 4 (6), 525–536. https://doi.org/10.1016/S2213-8587(15)00482-9.

Novo Nordisk, A./S.. OZEMPIC highlights of prescribing information 2017. Available at, Accessed June 2021). https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209637lbl.pdf.

Novo Nordisk, A./S.. SAXENDA EPAR summary for the public. Available at, (Accessed June 2021). https://www.ema.europa.eu/en/documents/overview/saxenda-epar-summary-public_en.pdf.

Novo Nordisk, A./S.. SAXENDA highlight of prescribing information. Available at, (Accessed June 2021). https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/206321s007lbl.pdf.

O'Neil, P.M., Birkenfeld, A.L., McGowan, B., Mosenzon, O., Pedersen, S.D., Wharton, S., Wilding, J.P.H., 2018. Efficacy and safety of semaglutide compared with liraglutide and placebo for weight loss in patients with obesity: a randomised, double-blind, placebo and active controlled, dose-ranging, phase 2 trial. Lancet 392 (10148), 637–649. https://doi.org/10.1016/s0140-6736(18)31773-2.

Reed, J., Bain, S., Kanamarlapudi, V., 2020. Recent advances in understanding the role of glucagon-like peptide 1. F1000Res. https://doi.org/10.12688/f1000research.20602.1, 9.

Sharma, D., Verma, S., Vaidya, S., Kalia, K., Tiwari, V., 2018. Recent updates on GLP-1 agonists: current advancements & challenges. Biomed. Pharmacother. 108, 952–962. https://doi.org/10.1016/j.biopha.2018.08.088.

Sheahan, K.H., Wahlberg, E.A., Gilbert, M.P., 2020. An overview of GLP-1 agonists and recent cardiovascular outcomes trials. Postgrad. Med. 96 (1133), 156–161. https://doi.org/10.1136/postgradmedj-2019-137186.

Strohl, W.R., 2015. Fusion proteins for half-life extension of biologics as a strategy to make biobetters. BioDrugs 29 (4), 215–239. https://doi.org/10.1007/s40259-015-0133-6.

Trainor, G.L., 2007. The importance of plasma protein binding in drug discovery. Expet Opin. Drug Discov. 2 (1), 51–64. https://doi.org/10.1517/17460441.2.1.51.
U.S. Food and Drug Administration. FDA news release. https://www.fda.gov/news-events/press-announcements/fda-approves-new-drug-treatment-chronic-weight-management-first-2014.
Zheng, Y., Ley, S.H., Hu, F.B., 2018. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat. Rev. Endocrinol. 14 (2), 88–98. https://doi.org/10.1038/nrendo.2017.151.