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Glutazumab, a novel long-lasting GLP-1/anti-GLP-1R antibody fusion protein, exerts anti-diabetic effects through targeting dual receptor binding sites

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ABSTRACT

Aims: Glucagon like-peptide-1 (GLP-1)-based drugs have been proposed as mono- or combined therapy for type 2 diabetes mellitus. Thus we characterized a novel antibody fusion protein engineered by linking the human GLP-1 derivative to a humanized GLP-1 receptor (GLP-1R) antibody *via* a peptide linker.
Materials and methods: Glutazumab was characterized by receptor binding and reporter activation assays, and its specificity was investigated with the aid of the cognate receptor antagonist exendin (9-39) and antibody Ab1. Pharmacokinetics was evaluated in Sprague-Dawley (SD) rats and cynomolgus monkeys, and pharmacodynamics was assessed in normal ICR and spontaneous type 2 diabetic KKAy mice. Hypoglycemic effects were evaluated after acute administration and glucose metabolism and β-cell function were assessed with repeated administrations. Dulaglutide was a positive control in all experiments.
Results: Glutazumab significantly bound and activated GLP-1R, but the receptor antagonist exendin (9-39) did not inhibit the activation except when combined with Ab1. Single injection of glutazumab reduced the blood glucose in ICR mice and KKAy mice, and the half-lives in SD rats and cynomolgus monkeys were 18 h and 33.6 h. Repeated injections of glutazumab controlled glycemic fluctuations and improved β-cell function in KKAy mice.
Conclusions: As a novel GLP-1R agonist, glutazumab may be a potential treatment for T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by insulin resistance and progressive β-cell failure. To compensate insulin resistance, β cells must secrete increasingly more insulin to maintain glucose homeostasis and this causes metabolic stress and deterioration, loss of glycemic control and the onset of T2DM. Therefore, restoration of β-cell function is crucial to the treatment of T2DM [1]. Obesity is a major risk factor for onset of T2DM, while hypoglycemia is a common adverse effect of T2DM treatment with insulin and sulphonylureas and has important consequences on cognitive function [2,3], so T2DM drugs that inhibit weight gain and promote stable blood glucose are ideal.

Glucagon-like peptide-1 (GLP-1) is an important endogenous incretin for glucose metabolism and a novel treatment for T2DM [4]. GLP-1 binds to GLP-1R to protect β cells from progressive failure by decreasing apoptosis and promoting proliferation, simultaneously enhancing insulin secretion upon glucose stimulation and suppressing appetite and gastric emptying without hypoglycemia or weight gain [5,6]. But natural GLP-1 is of little clinical utility because of rapid inactivation by dipeptidyl peptidase-IV (DPP-IV) and natural endogenous peptidase, as well as glomerular filtration. Therefore, it is essential to extend the half-life of GLP-1 for the development of GLP-1-based therapeutics. Five GLP-1 analogs in the US and six in Europe have been approved [7]. Fusing GLP-1 to a large “carrier” moiety is an effective method to extend half-life and dosing interval. Albiglutide is a DPP-IV resistant GLP-1 dimer fused to human albumin with a half-life of four to seven days, and dulaglutide is a DPP-IV resistant GLP-1 analog fused to a modified immunoglobulin G (IgG4) Fc fragment. Both drugs are injected once weekly [8,9].

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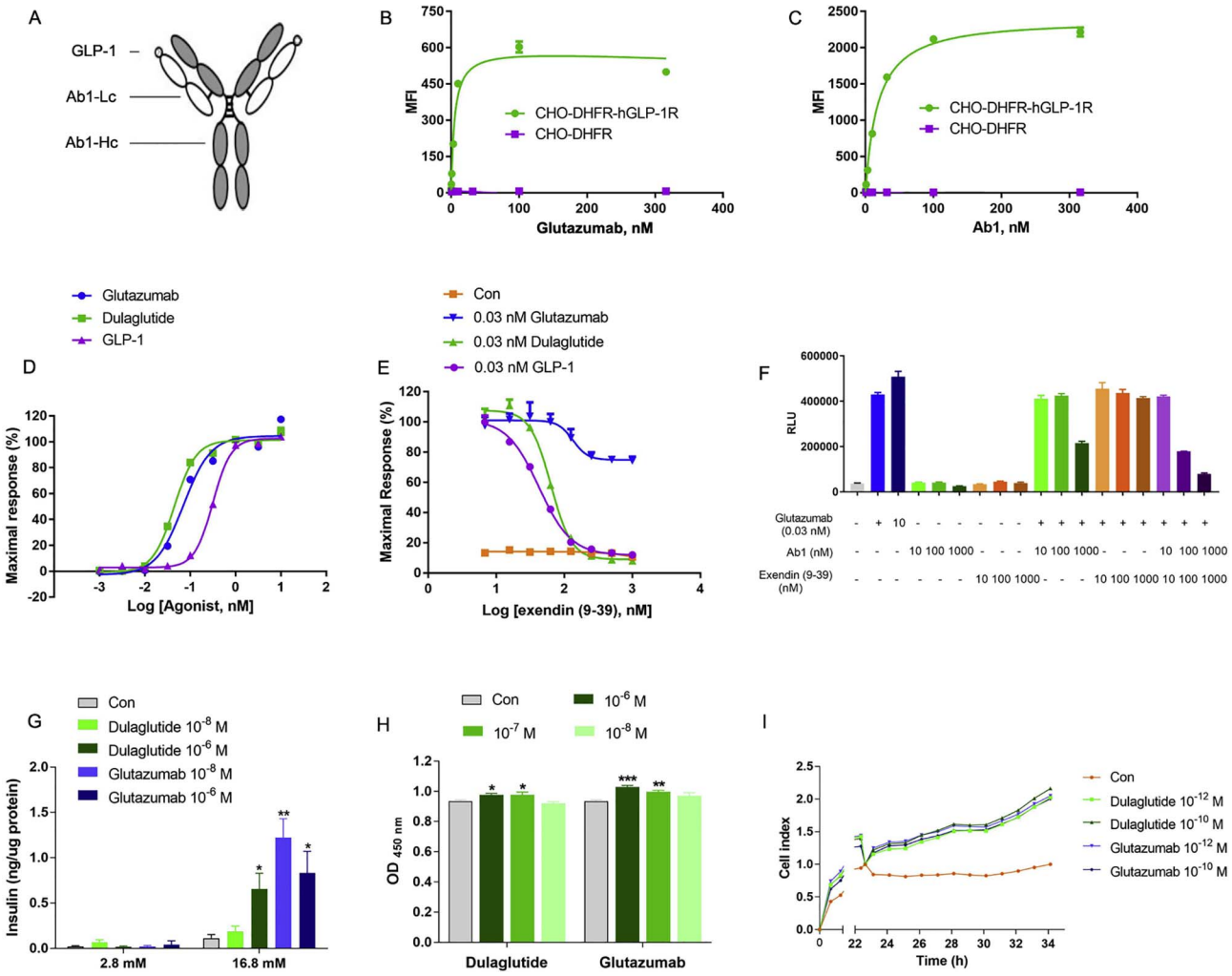

Fig. 1. Glutazumab enhanced glucose-stimulated insulin secretion and β-cell survival by specifically binding and activating GLP-1 receptor. A) Schematic diagram of glutazumab, B) Binding of glutazumab to human GLP-1 receptor (hGLP-1R), C) Binding of Ab1 to hGLP-1R, D) Luciferase activity induced by glutazumab, dulaglutide and natural GLP-1, E) Inhibition of the luciferase activity by exendin (9-39), an antagonist of GLP-1R, F) Inhibition of the luciferase activity by exendin (9-39), Ab1 or the combination of exendin (9-39) and Ab1, G) Insulin secretion induced by glutazumab and dulaglutide with 2.8 and 16.8 mM glucose, H) NIT-1 cell viability induced by glutazumab and dulaglutide, I) Real-time NIT-1 cell growth . All data were expressed as means ± SEM, n = 3. For G and H, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* Con.

Here we describe glutazumab, a novel GLP-1R agonist that is constructed by fusing a DPP-IV-resistant GLP-1 (7-35) fragment to the light chains of humanized GLP-1R antibody (IgG2) via a 23-amino acid peptide linker. Glutazumab possesses pharmacological effects of the natural GLP-1 and the structural attributes of a highly specific antibody (Fig. 1A), and its pharmacology and pharmacokinetics were fully characterized.

2. Materials and methods

2.1. Materials

Geneticin, sodium azide, pentobarbital sodium, exendin (9-39), penicillin, streptomycin, streptavidin, bovine serum albumin (BSA) and HRP conjugated goat anti-human IgG Fc were obtained from Sigma-Aldrich (St. Louis, MO, USA). DMEM/F12, fetal bovine serum (FBS) and dialyzed FBS were purchased from Gibco (Rockville, MD, USA). CHO-DHFR and NIT-1 cells were obtained from Cell Resource Center of Shanghai Institute for Biological Sciences (Shanghai, China) and ATCC (Manassas, VA, USA) respectively. The steady-Glo luciferase assay system and pEXP-TF and pGL3 vectors were obtained from Promega (Madison, WI, USA). The DyLight 488 NHS ester was purchased from Thermo Fisher Scientific (Waltham, MA, USA). BCA assay kit was obtained from Applygen Technologies (Beijing, China). GLP-1 (7-37) and dulaglutide were purchased from Phoenix Pharmaceuticals (Belmont, CA, USA) and Eli Lilly (Indianapolis, IN, USA) respectively. DPP-IV inhibitor was obtained from Millipore (Darmstadt, Germany). Acetaminophen was purchased from National Institutes for Food and Drug Control (Beijing, China). The biotinylated antibody recognizing the N-terminus of GLP-1 was supplied by Gmax Biopharm (Hangzhou, China). CCK-8 assay reagent was purchased from Dojindo Laboratory (Kumamoto, Japan). Insulin (mouse) ultrasensitive ELISA kit was bought from Alpco (Salem, NH, USA). HbA1c assay kit was bought from Homa Biological (Beijing, China). Glucose assay kit was obtained from Biosino Bio-Technology & Science Inc. (Beijing, China). Fluorescence-activated cell sorting (FACS) analysis was performed on Guava of Merck KGaA (Darmstadt, Germany). The iCELLigence Real Time Cell Analyser system and the 8-well E-plates were purchased from ACEA Biosciences Inc. (Hangzhou, China). Glucose, saline and reagents for preparing KRH and PBS buffers were obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China).

2.2. Construction of plasmids and stable cell lines

The human GLP-1 receptor (hGLP-1R) cDNA was amplified and cloned into pEXP-TF vector, and the reporter plasmid was constructed