Generation of a High-Affinity, Humanized Monoclonal Antibody (mAb) That Effectively Neutralizes Glucose-Dependent Insulinotropic Polypeptide (GIP)

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Abstract

Introduction

- GIP, a peptide hormone synthesized in intestinal K-cells, is both insulinotropic and insulin mimetic and accordingly plays a critical role in promoting nutrient uptake and storage. We recently developed a mouse mAb (mmAb) and reported that this mmAb decreased weight gain in C57BL/6 mice fed a high-fat diet by nearly 50%, without affecting food intake. The aim of this study was to "humanize" our GIP mmAb by grafting its complementary determining regions onto a human IgG "scaffold" and to characterize its binding characteristics.

Methods

- Surface plasmon resonance was performed to determine the binding affinity of GIP to the humanized GIP mAb (hmAb). A reporter cell line expressing the GIP receptor, which is activated by GIP in a concentration-dependent manner, was used in a modified Schild's assay to demonstrate mAb-dependent GIP neutralization. Next, GIP hmAbs were administered ip, and blood samples were collected over 2 weeks, and peak plasma concentration (Cmax), and T1/2 were determined. Finally, a recently created tool was used to calculate the "humanness score" of our derived GIP hmAb. This score represents the degree to which the mAb's variable region possesses human-like characteristics.

Results

- The binding affinity (KD) of the GIP hmAb was calculated to be 0.9 nM, which is greater than the original GIP mmAb KD of 3.3 nM. Similar to the original mmAb, GIP hmAbs neutralized GIP signaling in vitro in a concentration-dependent manner. The Schild's assay plot showed human and mouse mAb equilibrium dissociation constants of 2.2 µM and 3.2 µM, respectively, indicating similarly that the GIP hmAb binds more avidly to GIP than the mmAb. The calculated Cmax values for the 10 and 30 mg/kg BW doses of GIP mAb were 10.12± 0.7 µg/ml and 32.6±1.0 µg/ml, respectively, and the T1/2 was determined to be ~7-10 days for both doses. Finally, the variable heavy and light chain (VL) humanness scores that were used to create the hmAb were 75.81 and 84.86, respectively, indicating human-like properties of this GIP mAb.

Discussion

- A GIP hmAb was shown to bind GIP with higher affinity than the mmAb, and similarly the hmAb more potently inhibited GIP signalling in vitro than the original GIP mmAb. Furthermore, the calculated T1/2 for the GIP hmAb in vivo is comparable to other biological agents, and along with its excellent humanness score, indicate that this mAb could represent an effective biological agent for treating obesity and related disorders in humans

Introduction

- In a separate study, PBS or GIP mAbs (60 mg/kg BW/week) were injected ip to ob/ob mice fed normal mouse chow for 8 weeks, and body weight and food consumption were measured weekly. Glucose-dependent insulinotropic polypeptide (GIP) was initially isolated from porcine small intestine in 1969 and was named "gastric inhibitory polypeptide" for its ability to inhibit acid secretion.
- In addition to its gastric inhibitory effects, subsequent investigation demonstrated that in the presence of glucose, GIP enhanced insulin release by pancreatic islet β-cells. It was, therefore, suggested that GIP may function as an "incretin," a proposed enteric factor that stimulates insulin release and that plays a physiological role in maintaining glucose homeostasis.
- GIP is also insulin mimetic and thereby plays a critical role in promoting nutrient uptake and storage. A recent study demonstrated that GIP promotes fat accumulation in humans. All of these metabolic properties support the notion that GIP represents a major factor contributing to the development of insulin resistance and obesity.
- GIP- and GIPR-null mice fed a high-fat diet are resistant to the development of obesity and related co-morbidities.
- High-fat fed mice treated with a mouse anti-GIP monoclonal antibody are also resistant to the development of obesity and related co-morbidities.
- We used the mouse anti-GIP monoclonal antibody sequence to generate humanized derivatives, and the characterization of the humanized anti-GIP monoclonal antibodies, including a lead drug candidate are presented below.

Methods & Materials

- ***Evaluating GIP-specific mAbs by ELISA***. MaxiSorp plates were coated with synthetic mouse hGIP-(1–42) using 50 µl/well of a 4 µg/ml solution in 0.2 M carbonate-bicarbonate buffer, pH 9.4. After incubation overnight at 4°C, wells were washed with PBS before mAb solutions were added for 2 h at 37°C. The solutions were then aspirated and the wells washed with PBS before a solution of goat antihuman IgG (H+L)-HRP(Southern Biotech) diluted 1:1000 in 0.4 mg/ml BSA in PBS was added. After incubation for 1 h at 37°C, the solution was removed, and the wells were washed before HRP content was detected using a 4 mg/ml solution of o-phenylenediamine dihydrochloride in 0.4 mg/ml urea hydrogen peroxide, and 0.05 M phosphate-citrate, pH 5.0. The reaction was stopped by the addition of an equal volume of 4 N sulfuric acid, and HRP activity was quantified by measuring absorbance at 490 nm.
- ***Identification of mAbs that specifically neutralize GIP using a cell culture model.*** To demonstrate that GIP mAbs specifically neutralized GIP activity, purified IgG at concentrations of 0, 1.56, 3.13, 6.25, 12.5, 25 or 50 µg/ml were mixed with increasing amounts (0, 0.1, 0.2, .05, 1.0, 10.0 and 100.0 nM) of hGIP in DMEM containing 5% FBS. After incubation at 37°C for 15 min, the mixtures were added to reporter cells. These reporter cells, which respond to both GIP by increasing LacZ gene expression, were derived by the transduction of LVIP cells with lentiviral pseudoparticles containing cDNAs encoding the mouse GIPR (mGIPR) receptor. The cells were incubated for 4 h at 37°C before the mixtures were removed, and the cells were then washed, and the reporter activity was assayed.
- ***Affinity determination by surface plasmon resonance.*** Binding experiments were performed using a Biacore 3000 instrument with CM5 sensor chips (GE Healthcare Life Sciences). The purified mAb (ligand) was immobilized using an amine couple kit. Human GIP (analyte) solutions from 0.05 to 1,000 nM were prepared in 0.01 M HEPES, pH 7.4, 0.15 M NaCl with 0.005% (vol/vol) Surfactant P20. Each injection was set for 200 s at the flow rate of 20 µl/min for binding with a dissociation time of 300 s. All experiments were performed at 25°C. At the end of each cycle, glycine-HCl, pH 3.0, was used for regeneration. The sensograms were analyzed by BIAevaluation 3.0 package. The equilibrium dissociation constants (KD), defined as the k_d/k_a ratio (Ka, acid association rate; KD, equilibrium dissociation constant), were then calculated.
- ***Pharmacokinetic analysis – Determining the circulating T1/2 of biologically active mAb***. 9-week-old C57BL/6 mice (n= 4 per group) were administered either 10 or 30 mg/kg BW mAb by ip injection. Two 5-µl aliquots of blood were collected at times: 1, 2, 4, 8 12, 24, 48, 96, 168 and 336 h. The aliquots were immediately diluted with 45 µl 10 mM EDTA, centrifuged at 3000 x g for 2 min and stored frozen at -20°C. The amount of mAb in each sample capable of binding to GIP (the bioavailable fraction of mAb) was measured using the ELISA described above.

Results

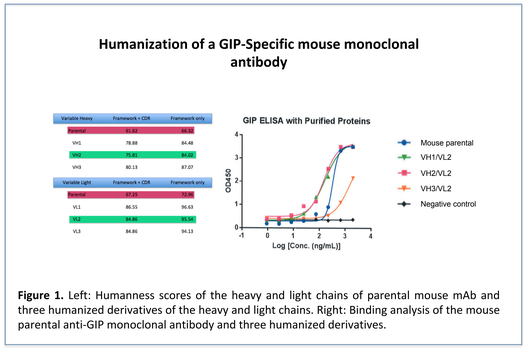

Figure 1. Left: Humanness scores of the heavy and light chains of parental mouse mAb and three humanized derivatives of the heavy and light chains. Right: Binding analysis of the mouse parental anti-GIP monoclonal antibody and three humanized derivatives.

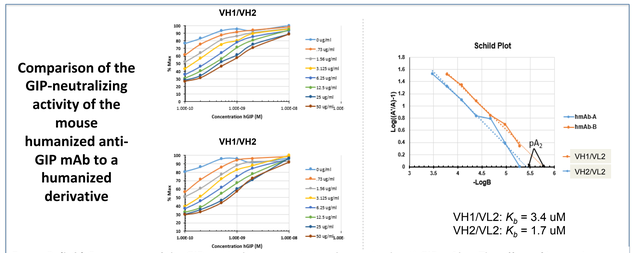

Figure 3. (left) Comparison of the GIP-neutralizing activity two humanized anti-GIP mAbs. The effect of increasing concentrations of humanized anti-GIP on hGIP dose-response experiments using GIP-responsive reporter cells in vitro. **(Right) Estimation of the equilibrium dissociation constants (K_B) of the two hmAbs by Schild plot.** [A] is the hGIP (agonist) concentration to give an effect in the absence of hmAb (antagonist). [A'] is the hGIP concentration to give a certain effect in the presence of the hmAb. [pA2] indicates the location at which the linear regression line intercepts the X-axis, which is used to estimate K_B.

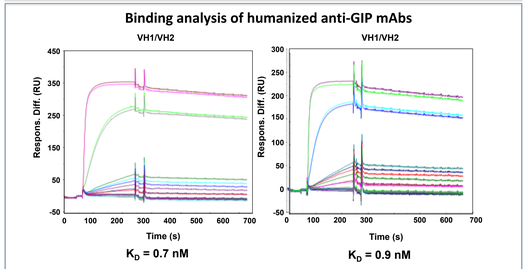

Figure 2. Binding of analyte hGIP to humanized mAbs (ligands) was detected by surface plasmon resonance (SPR). Representative association and dissociation sensograms from binding analysis are shown. The equilibrium dissociation constants (K_D), defined as the k_d/k_a ratio (K_a, acid association rate; K_D, equilibrium dissociation constant), were calculated using Biacore software.

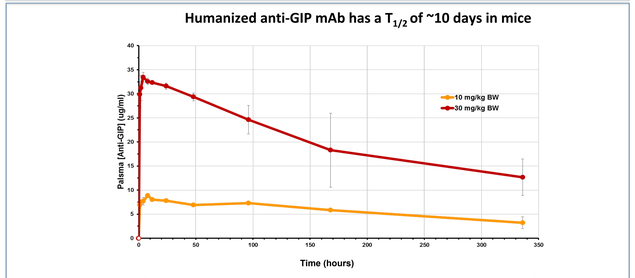

Figure 4. Pharmacokinetic analysis of humanized GIP mAb in mice. Plasma levels of humanized GIP mAb were measured by ELISA at various times after *ip* administration of humanized GIP mAb at doses 10 mg/ kg BW (orange) or 30 mg/kg BW (red), respectively. Plasma mAb concentrations (µg/ml) versus time in hours are plotted above. The specific ELISA identifies bioavailable mAb.

Discussion & Summary

- Using amino acid sequences from a mouse anti-GIP monoclonal antibody as a guide, three humanized heavy chain and three light chain derivatives were generated. When different combinations of the derived light chains and heavy chains were expressed in HEK 293 cells, only mAbs possessing the VL2 derivative were able to demonstrate specific binding to hGIP in an ELISA.
- Protein A purified humanized mAb variants VH1/VL2 and VH2/VL2 possessed affinities for hGIP higher than the parental mouse mAb when evaluated by ELISA. The variant VH3/VL2 bound only weakly to GIP in the same assay.
- Using SPR, the humanized mAbs were zshown to have higher affinities for hGIP than the parental mAb (0.7 nM and 0.9 nM for VH1/VL2 and Vh2/VL2, respectively, compared to 3.3 nM for the parental mouse mAb).
- When evaluated in a cell cultured based assay, the two humanized mAbs, VH1/VL2 and VH2/L2, were demonstrated to specifically neutralize hGIP in vitro in a dose-dependent manner. Using a modified Schild's assay, the two humanized mAbs were demonstrated to neutralize hGIP with similar potencies (KB = 3.4 µM and KB 1.7 µM, for VH1/VL2 and VH2/VL2, respectively).
- Interestingly, the VH2/VL2 variant which had a lower affinity for hGIP based on SPR data, was more effective in neutralizing hGIP in the cell culture model.
- As the humanized mAb VH2/VL2 more effectively neutralized hGIP, it was chosen for further development and is the precursor to the drug candidate NM-136.
- Pharmacokinetic analysis demonstrated that VH2/VL2 had a circulating T1/2 of ~10 days in mice.

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