

Novel Platform for Predicting Drug Effects in Patients with Acromegaly: Translational Exposure-Response Evaluation of Growth Hormone-Inhibitory Effect of Octreotide after Growth Hormone-Releasing Hormone Stimulation

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ABSTRACT

Acromegaly is a chronic systemic disease characterized by facial and peripheral changes caused by soft tissue overgrowth and is associated with multiple comorbidities. Despite available surgical and medical therapies, suitable treatments for acromegaly are still lacking. Efficient drug development requires an understanding of the exposure-response (E-R) relationship based on nonclinical and early clinical studies. We aimed to establish a platform to facilitate the development of novel drugs to treat acromegaly. We evaluated the E-R relationship of the growth hormone (GH)-inhibitory effect of the somatostatin analog octreotide under growth hormone-releasing hormone + arginine stimulation in healthy participants and compared the results with historical data for patients with acromegaly. This randomized five-way crossover study included two placebo and three active-treatment periods with different doses of octreotide acetate. GH secretion in the two placebo periods was comparable, which confirmed the reproducibility of the response with no carryover effect. GH secretion was inhibited by low-, medium-, and high-dose octreotide acetate in a dose-

dependent manner. We also examined the E-R relationship in monkeys as a preclinical drug evaluation study and in rats as a more convenient and simple system for screening candidate drugs. The E-R relationships and EC₅₀ values were similar among animals, healthy participants, and patients with acromegaly, which suggests that GH stimulation studies in early research and development allowed simulation of the drug response in patients with acromegaly.

SIGNIFICANCE STATEMENT

This study demonstrated similar exposure-response relationships in terms of the growth hormone-inhibitory effect of octreotide after growth hormone-releasing hormone stimulation among healthy participants, monkeys, and rats. The research methods and analyses utilized in this study will be useful for simulating the dosages and therapeutic effects of drugs for acromegaly and will facilitate the research and development of novel therapeutic agents with similar modes of action.

Introduction

Acromegaly is a chronic systemic disease characterized by facial and peripheral changes caused by soft tissue overgrowth. Acromegaly is also associated with multiple complications, such as diabetes mellitus, heart failure, and arterial hypertension, and is associated with increased mortality if not adequately treated (Gadelha et al., 2019). Acromegaly is caused by the hypersecretion of growth hormone (GH), usually as a result of the presence of a benign pituitary adenoma (Melmed, 2009).

Acromegaly is considered a rare disease and has a population prevalence of 40–70 per million and an incidence of 3–4 per million per year (Chanson and Salenave, 2008). The mortality rate can be reduced to general population levels by reducing GH levels and normalizing levels of insulin-like growth factor-1 (IGF-1) (Holdaway et al., 2004).

The primary treatment after a diagnosis of acromegaly involves surgery to remove the pituitary adenoma and follow-up medical treatment with somatostatin analogs (SSAs) for patients who do not achieve biochemical control after surgery (Giustina et al., 2020). However, some patients fail to respond to octreotide or lanreotide, whereas pasireotide carries a risk of hyperglycemia (Gadelha et al., 2014). These SSAs are administered as monthly intramuscular or deep subcutaneous

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ABBREVIATIONS: AUC, area under the concentration-time curve; E₀, GH AUC after treatment with placebo or vehicle; E_{max}, maximum inhibitory effect; E-R, exposure-response; GH, growth hormone; GHRH, GH-releasing hormone; Hill, Hill coefficient; IGF-1, insulin-like growth factor-1; OFV, objective function value; SSA, somatostatin analog; sst, somatostatin receptor.

injections, and lifelong injections of these long-acting SSAs impose a significant burden on the functioning, well being, and daily lives of patients with acromegaly (Strasburger et al., 2016). In 2020, the US Food and Drug Administration (FDA) approved the use of oral octreotide capsules; however, these capsules should be administered carefully before meals to ensure adequate oral absorption (Fleiseriu et al., 2021). Thus, despite available surgical and medical therapies, additional treatments for acromegaly are still required.

Clinical trials of novel somatostatin receptor agonists in patients with acromegaly are generally difficult because of the small number of patients. It is an expectation of regulatory authorities that analyses of the exposure-response (E-R) relationship and subsequent simulations of clinical responses are expected to contribute to the estimation of the appropriate dosage in the event of limited clinical study results. These analyses can also help researchers avoid unnecessary clinical studies to develop medicinal products targeting populations and diseases for which clinical studies are not feasible, such as orphan diseases like acromegaly (FDA, 2003; PMDA, 2020). It is therefore important to establish a clear understanding of the E-R relationship in healthy participants before proceeding to studies in patients in order to enable efficient drug development while reducing the scale of subsequent clinical trials.

The effects of SSAs on GH and IGF-1 as the clinical endpoints of acromegaly have been reported in healthy participants. However, it takes weeks to months for IGF-1 to reach steady state after the administration of SSAs (Tiberg et al., 2015). Additionally, although GH is a useful biomarker that responds instantaneously to a single dose of SSA, it is secreted irregularly in a pulsatile manner throughout the day, varies greatly among individuals, and cannot be controlled by SSAs (Dimaraki et al., 2001, 2003). Transient stimulation of GH levels by the administration of growth hormone-releasing hormone (GHRH) can thus be used to confirm the GH-inhibitory effect of SSAs while mitigating the effects of diurnal variation in healthy participants (Tuvia et al., 2012; Golor et al., 2012). Various methods can be used to stimulate GH using this approach, which is also used to diagnose GH deficiency (Ho, 2007), and the ability of this method to demonstrate an E-R relationship that reflects the drug response in patients with acromegaly is unclear.

This study was aimed to develop a novel platform for predicting drug effects in patients with acromegaly by translational evaluation of the E-R in terms of the GH-inhibitory effect of drugs in healthy participants and animals after GH stimulation. We used the gold-standard SSA for the treatment of acromegaly, octreotide acetate (Sandostatin), as a model drug; analyzed the E-R relationship of its GH-inhibitory effect of under GHRH + arginine stimulation in healthy participants; and compared the results with historical data for patients with acromegaly (FDA, 1998). We also conducted similar studies in normal monkeys and rats to compare the E-R relationships with that in humans.

Materials and Methods

Ethics. All *in vitro* and *in vivo* studies were conducted in accordance with the “Safety Control Regulations for Pathogens,” “Efficacy Pharmacology: Standards for Reliance,” and “Guidance for Animal Experiments” established by Ono Pharmaceutical Co., Ltd. The clinical study was conducted in accordance with the study protocol;

International Conference on Harmonization Good Clinical Practice guidelines; US Code of Federal Regulations Title 21, Parts 50, 56, and 312; local laws and regulations; and the ethical principles of the Declaration of Helsinki. All participants were required to read, sign, and date informed consent forms summarizing the discussion prior to screening. The study protocols were reviewed and approved by an independent institutional review board.

***In Vitro* Agonistic Effects on Somatostatin Receptor 2.** The somatostatin receptor (sst) family is divided into five subtypes (sst1–5), and the GH-inhibitory effect of octreotide is mainly based on the activation of sst2 (Schmid and Schoeffer, 2004). We conducted a preliminary *in vitro* study to confirm the agonistic effects of octreotide on rat, monkey, and human sst2 using cAMP inhibition assays in CHO-K1 cells stably expressing each sst2. The cell lines were treated with 10 $\mu\text{mol/l}$ forskolin and various concentrations of octreotide or somatostatin (active control) at 37°C for 30 minutes, after which intracellular cAMP concentrations were determined by ELISA. A nonlinear regression analysis of the concentration-response curves was performed using GraphPad Prism version 5.04 (GraphPad Software, San Diego, CA), and the EC_{50} value and 95% confidence interval were estimated for each substance.

***In Vivo* GH Stimulation Studies.** *In vivo* GH stimulation studies were carried out in humans, monkeys, and rats (Fig. 1), as detailed below.

Clinical Study in Healthy Participants. We conducted a single-center, double-blind, randomized, placebo-controlled, five-way crossover study to evaluate the GH response after infusion of octreotide acetate or placebo after combined administration of GHRH and arginine. The study included 24 healthy adult males aged 18–40 years with a body mass index ≥ 18.5 and $< 25 \text{ kg/m}^2$. Participants with fasting blood glucose outside the normal range were excluded from the study. Octreotide acetate was supplied as sterile 50- $\mu\text{g/ml}$ ampules of Sandostatin injection (Novartis, Basel, Switzerland). The dose was selected based on available information on the pharmacokinetics of octreotide in healthy participants. Octreotide exposure linearly correlates with the dosage, and the elimination half-life is about 90–110 minutes (total clearance is about 9.6 l/h) (Chanson et al., 1993). The doses selected were chosen to cover the effective concentrations in patients with acromegaly in order to ensure a dose range sufficient to assess the E-R relationship. Each dose of octreotide acetate was diluted in sodium chloride 0.9%, USP for injection (low dose, 8.5 $\mu\text{g}/200 \text{ ml}$; medium dose, 21 $\mu\text{g}/200 \text{ ml}$; high dose, 92 $\mu\text{g}/200 \text{ ml}$) and infused intravenously at a rate of 25 ml/h over approximately 8 hours. Placebo was supplied as sterile sodium chloride 0.9%, USP for injection with equivalent volume and infusion time.

The study comprised two placebo periods and three active-treatment periods with different doses of octreotide acetate. The use of two placebo periods allowed the assessment of the reproducibility of GHRH + arginine stimulation, and the use of three different dose levels of octreotide acetate generated sufficient data to construct a robust E-R model in healthy participants. Each participant received placebo in the first fixed treatment period (placebo 1) and was then randomized to one of four treatment sequences (placebo 2-low-medium-high, low-high-placebo 2-medium, medium-placebo 2-high-low, or high-medium-low-placebo 2) to minimize assignment bias. The participants underwent a washout period of at least 7 days between the start of each octreotide acetate/placebo infusion. Either octreotide acetate or placebo was infused for 8 hours starting at 8:00 AM on day 1 of each treatment period. An intravenous bolus of GHRH (GHRH Ferring, Ferring Pharmaceuticals Ltd., Middlesex, UK) of 1 $\mu\text{g/kg}$ (maximum dose 100 μg) was administered 6 hours after octreotide acetate/placebo administration to stimulate GH, which was followed by a 30-minute infusion of 30 g L-arginine hydrochloride (R-Gen 10; Pfizer Inc., New York, NY) in 300 ml saline. The participants fasted overnight for at least 10 hours prior to the start of the octreotide acetate/placebo infusion and for 3 hours after the start of the infusion, after which they were required to consume a standard meal with a low-to-moderate fat content within 30 minutes. The participants were

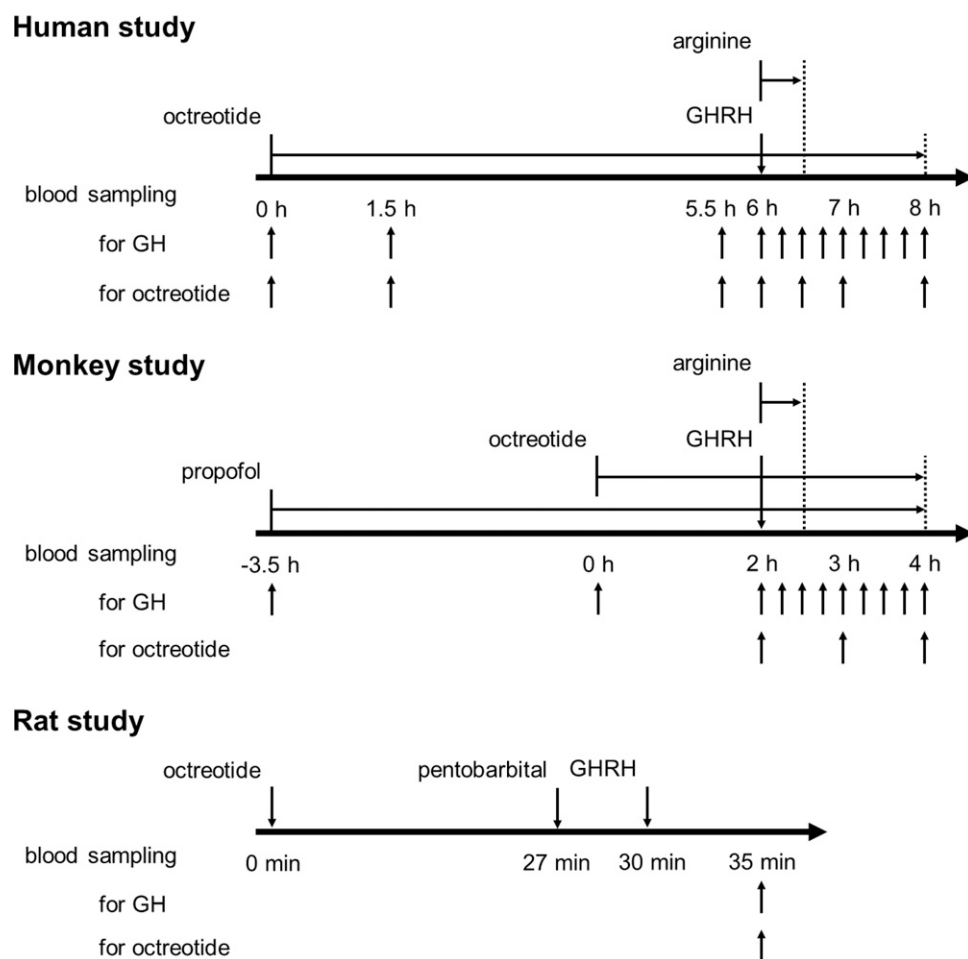


Fig. 1. Schedule of in vivo studies.

therefore partially fasted prior to the combined administration of GHRH and arginine. Blood samples were drawn at 0, 1.5, 5.5, 6, 6.5, 7, and 8 hours after starting octreotide administration for serum octreotide measurements and at 0, 1.5, 5.5, 6, 6.25, 6.5, 6.75, 7, 7.25, 7.5, 7.75, and 8 hours after starting octreotide administration for GH measurements.

In Vivo Study in Monkeys. Eight male cynomolgus monkeys (3–6 years old; Eve Bioscience, Wakayama, Japan and GMJ, Hyogo, Japan) were used in this study. The study consisted of four periods in which each animal received vehicle or low-, medium-, or high-dose octreotide with a washout period of at least 7 days between the start of each octreotide/vehicle infusion. On the first day of each period, monkeys were anesthetized by an intravenous bolus of propofol (Propofol Intravenous Injection 2% Maruishi; Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) 3.0 mg/kg, which was followed by intravenous infusion of propofol at the appropriate infusion rate for each animal. An additional bolus of propofol was administered as needed to adjust the depth of anesthesia. Each dose of octreotide (GenScript, Piscataway, NJ) was selected based on an in-house preliminary pharmacokinetic study. After a single intravenous bolus of octreotide (0.3 mg/kg), the plasma octreotide concentration disappeared rapidly with an elimination half-life of 2.80 hours (unpublished data). A loading dose of octreotide with a different infusion rate was used in monkeys to avoid overdose of propofol. Specifically, each dose of octreotide or vehicle (saline) was infused intravenously over 4 hours, which was approximately 3.5 hours after starting the propofol administration. The infusion rate was varied between the first 2 hours and the subsequent 2 hours to reach steady state within 2 hours and to maintain a steady plasma octreotide concentration during GH stimulation (low dose,

0.233 → 0.133 $\mu\text{g}/\text{kg}$ per hour; medium dose, 0.467 → 0.27 $\mu\text{g}/\text{kg}$ per hour; high dose, 0.7 → 0.4 $\mu\text{g}/\text{kg}$ per hour). Two hours after treatment with octreotide/vehicle, an intravenous bolus of GHRH (GRF Sumitomo for injection 100; Dainippon Sumitomo Pharma, Osaka, Japan) (30 $\mu\text{g}/\text{kg}$) was administered, which was followed by a 30-minute intravenous infusion of L-arginine hydrochloride (Kishida Chemical, Osaka, Japan) (5 g/kg per hour) to stimulate GH secretion. Blood samples were drawn at 2, 3, and 4 hours after starting octreotide administration for octreotide measurements and at 0, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, and 4 hours after starting octreotide administration for GH measurements. The collected blood samples were centrifuged at $12,000 \times g$ for 5 minutes at 4°C , and the supernatant was collected as plasma. The plasma was cryopreserved at -80°C until measurement of GH and octreotide concentrations.

In Vivo Study in Rats. Male Crl:CD (Sprague Dawley) IGS rats (6 weeks old; Charles River Laboratories Japan, Kanagawa, Japan) were used in this study. In a preliminary pharmacokinetic study to determine the dosing schedule after a single subcutaneous bolus of octreotide (GenScript, 1 $\mu\text{g}/\text{kg}$), the plasma octreotide concentrations reached maximum 15 minutes after administration with an elimination half-life of 0.694 hours (unpublished data). Based on the result, octreotide (0.32, 0.65, 1.3, 2.2, or 6.5 $\mu\text{g}/\text{kg}$) or vehicle (saline) was administered subcutaneously to five rats in each dose group. At 27 minutes after octreotide/vehicle treatment, the rats were anesthetized with sodium pentobarbital (Kyoritsu Seiyaku, Tokyo, Japan) (50 mg/kg), which is known to decrease endogenous somatostatin secretion (Doi et al., 2004), injected into the tail vein, and at 30 minutes after octreotide/vehicle treatment, GHRH (Bachem AG, Bubendorf, Switzerland) (10 $\mu\text{g}/\text{kg}$) was injected into the tail vein to stimulate GH secretion.

Blood was then collected from the jugular vein 5 minutes later and centrifuged at $12,000 \times g$ for 5 minutes at 4°C , and the supernatant was collected as plasma. The plasma was cryopreserved at -20°C until measurement of GH and octreotide concentrations.

Bioanalytical Assessment of Octreotide. Plasma octreotide concentrations in the animal studies were determined by liquid chromatography/tandem mass spectrometry using a Nexera x2 system (Shimadzu Corporation, Kyoto, Japan) and Triple Quad 6500 mass spectrometer (AB Sciex, Framingham, MA), both of which were controlled by Analyst 1.5.2 software (AB Sciex). Chromatographic separation was performed using an Xbridge C18, $3.5 \mu\text{m}$, $2.1 \times 50 \text{ mm}$ column (Waters, Milford, MA) at a flow rate of 0.25 ml/min . The binary mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient was started at 20% B, increased to 60% B over 5 minutes, and then held at 60% B for 1 minute. The gradient was returned to 20% B immediately and equilibrated for 3 minutes before the next injection. The total cycle time for one injection was 9 minutes. The mass spectrometer was operated in the positive ionization mode using multiple reaction monitoring at a specific precursor ion \rightarrow product ion transition: mass-to-charge ratio $510.3 \rightarrow 120.0$.

Octreotide in plasma samples from the clinical study was assayed by Celerion (Lincoln, NE) using a similar validated method. Briefly, an aliquot of human plasma (EDTA) containing the analyte and internal standard was extracted using a solid-phase extraction procedure. The extracted samples were then analyzed by ultra-high performance liquid chromatography with a Triple Quad 6500 mass spectrometer using an electrospray ionization source. Positive ions were monitored in the multiple reaction monitoring mode. Quantitation was determined by weighted linear regression analysis of the peak area ratios of the analyte and internal standard.

Bioanalytical Assessment of GH. Human serum GH levels were measured by automated immunoassay (iSYS; Immunodiagnostic Systems IS-3700, Tyne and Wear, UK) using an automated analyzer IS-310400 (Immunodiagnostic Systems) and calibrated against the latest International Standard 98/574 (NIBSC, Hertfordshire, UK). Validation and characterization of the assay were performed according to the recommendations of the Clinical Laboratory Standards Institute, and the results have been published in detail elsewhere (Manolopoulou et al., 2012). Plasma GH concentrations in monkeys were measured using a human GH ELISA kit (Roche Diagnostics, Basel, Switzerland), and plasma GH concentrations in rats were measured using a Rat/Mouse Growth Hormone ELISA kit (EMD Millipore Corporation, Billerica, MA) according to the manufacturer's instructions. Absorbance was measured using a microplate reader (SpectraMax M5e; Molecular Devices, San Jose, CA).

Data Analysis. For samples with GH concentrations below the lower limit of quantitation, concentrations were set to one-half the lower limit of quantitation. Octreotide exposure was imputed as 0 after placebo/vehicle administration. E-R parameters were calculated by noncompartmental analyses using Phoenix WinNonlin (version 7.0; Certara USA Inc., Princeton, NJ). Area under the concentration-time curve (AUC) values were calculated by the linear up/log down trapezoidal method, and mean octreotide concentrations during GHRH stimulation were calculated by AUC at 2 hours divided by time. Data were expressed as mean \pm S.E.M. unless otherwise stated. One-way analysis of variance followed by Dunnett's test was applied to comparisons between the placebo/vehicle control group and test octreotide-treated groups using SAS 9.3 software (SAS Institute Inc., Cary, NC). $P < 0.05$ was considered statistically meaningful.

E-R Modeling. We investigated the relationship between octreotide exposure and the inhibition of GHRH-stimulated GH secretion using a nonlinear mixed-effects modeling approach. The pharmacodynamic endpoint for the human and monkey studies was the AUC at 2 hours of GH measured after the combined administration of GHRH and arginine, and the pharmacokinetic exposure was the mean octreotide concentration for 2 hours. We described the relationship using the maximum inhibitory effect (E_{max}) model as follows:

$$GH \text{ AUC} = E_0 \times \left(1 - \frac{E_{\text{max}} \times C^{\text{Hill}}}{EC_{50}^{\text{Hill}} + C^{\text{Hill}}} \right), \quad (1)$$

in which E_0 (GH AUC after treatment with placebo or vehicle) is the GH AUC after treatment with placebo/vehicle, E_{max} is the maximum inhibitory effect of octreotide on GH secretion, C is the mean plasma octreotide concentration, $Hill$ is the Hill coefficient describing the steepness of the E-R curve, and EC_{50} is the mean plasma octreotide concentration producing 50% effect of E_{max} . We developed the model using NONMEM (version 7.3.0; ICON Development Solutions, Ellicott City, MD). We tested interindividual variability for the parameters E_0 , E_{max} , and EC_{50} and assumed that the parameters followed a log-normal distribution. When interindividual variability was included in E_{max} , E_{max} was estimated using the logistic function ($E_{\text{max}} = e^{\text{logit}} / (1 + e^{\text{logit}})$) so that individual predicted E_{max} did not exceed 1, and interindividual variability was estimated for logit. E_{max} was fixed at 1 if it was considered close enough to 1. We described the residual error in all models using a proportional model. Model selection was guided by the objective function value (OFV) for nested models. We considered a decrease in OFV > 6.63 as statistically significant ($P < 0.01$, one degree of freedom) for the addition of one parameter.

We used the above equation to perform a similar analysis for the study in rats, using GH and octreotide concentrations at a certain time point as the E-R endpoint instead of GH AUC and mean octreotide concentration. We performed the E-R analyses using a naive-pooled method in which all data were analyzed together as if they were obtained from a single individual.

Results

In Vitro Agonistic Effects on sst2. The agonistic effects of somatostatin-14 and octreotide on CHO-K1 cells expressing recombinant sst2 receptors in each species are shown in Table 1. Octreotide inhibited forskolin-stimulated cAMP accumulation by approximately one order of magnitude more potently than somatostatin-14. Additionally, octreotide showed similar EC_{50} values in all species, which suggests that there were no interspecies differences in sst2 agonist activity.

Clinical Study in Healthy Participants. A total of 24 male participants (mean \pm S.D. of age, 28.3 ± 4.53 years; body mass index, $22.6 \pm 1.85 \text{ kg/m}^2$; and fasting blood glucose, $92.1 \pm 6.22 \text{ mg/dl}$) were initially randomized among four treatment groups ($n = 6$ per group). All three dose levels of octreotide acetate were well tolerated with no serious adverse events. All participants received five treatments per protocol, except for one participant who discontinued prior to

TABLE 1

Agonist effects of somatostatin-14 and octreotide for inhibition of forskolin-stimulated cAMP accumulation in CHO-K1 cells expressing rat, monkey, and human recombinant sst2 receptors. Data are mean EC_{50} values (95% confidence interval) from 3–4 experiments expressed as nmol/L.

Compound	Rat (n = 3)	Monkey (n = 4)	Human (n = 3)
Somatostatin-14	0.15 (0.12–0.17)	0.11 (0.082–0.14)	0.24 (0.19–0.30)
Octreotide	0.030 (0.026–0.034)	0.037 (0.030–0.047)	0.030 (0.026–0.035)

administration of octreotide acetate in period 5 and therefore did not receive the high-dose octreotide acetate.

The octreotide and GH concentration–time profiles and E-R relationship between GH AUC and mean octreotide concentration after GHRH + arginine stimulation are shown in Fig. 2. Octreotide acetate was administered as an 8-hour intravenous infusion, and a steady state was achieved by 6 hours after the start of the infusion and maintained over the subsequent GHRH + arginine stimulation study. Plasma octreotide concentrations after low-, medium-, and high-dose octreotide acetate administration increased in a dose-proportional manner, with mean ± S.E.M. levels 6–8 hours after the start of octreotide administration of 0.101 ± 0.00381 ng/ml after low-dose, 0.241 ± 0.00894 ng/ml after medium-dose, and 1.15 ± 0.0446 ng/ml after high-dose administration (Fig. 2A). The GHRH + arginine-stimulated secretion of GH was comparable after administration of placebo in fixed treatment period 1 (placebo 1) and in a randomized treatment period (placebo 2) (GH AUC 55.6 ± 4.27 ng × h/ml and 58.3 ± 5.46 ng × h/ml, respectively), which confirmed reproducibility of response with no carryover effect. The stimulated release of GH was significantly inhibited in a dose-dependent manner after administration of low-, medium-, and high-dose octreotide acetate (GH AUC 52.8 ± 4.68 ng × h/ml, $P = 0.790$; 41.3 ± 3.53 ng × h/ml, $P < 0.01$;

20.3 ± 2.52 ng × h/ml, $P < 0.001$; respectively) (Fig. 2B). In the final E-R model, the interindividual variability in E_{max} and E_0 was estimated, and E_{max} was estimated using the logistic function. Period effect tested as a covariate was not significantly incorporated. Observed values were well described by the model, and the estimated EC_{50} was 0.292 ng/ml (Fig. 2C).

In Vivo Study in Monkeys. A total of eight monkeys were treated in four periods. One monkey did not receive the medium dose because of an administration failure, thus the medium dose was evaluated in seven animals. Octreotide and GH concentrations versus time profiles and the E-R relationship between GH AUC and mean octreotide concentration after GHRH + arginine stimulation in monkeys are shown in Fig. 3. The mean plasma octreotide concentrations at 2–4 hours after the start of octreotide administration were 0.380 ± 0.0436 ng/ml after low-dose, 0.775 ± 0.0459 ng/ml after medium-dose, and 1.77 ± 0.162 ng/ml after high-dose octreotide, and they were maintained over the GHRH + arginine stimulation study (Fig. 3A). The plasma GH concentration before the start of anesthesia was 10.2 ± 1.86 ng/ml (unpublished data), and this decreased to 0.460 ± 0.106 ng/ml at the start of octreotide administration 3.5 hours after the start of anesthesia (Fig. 3B), which demonstrated reduced variations in baseline GH levels. The GH AUC after the combined

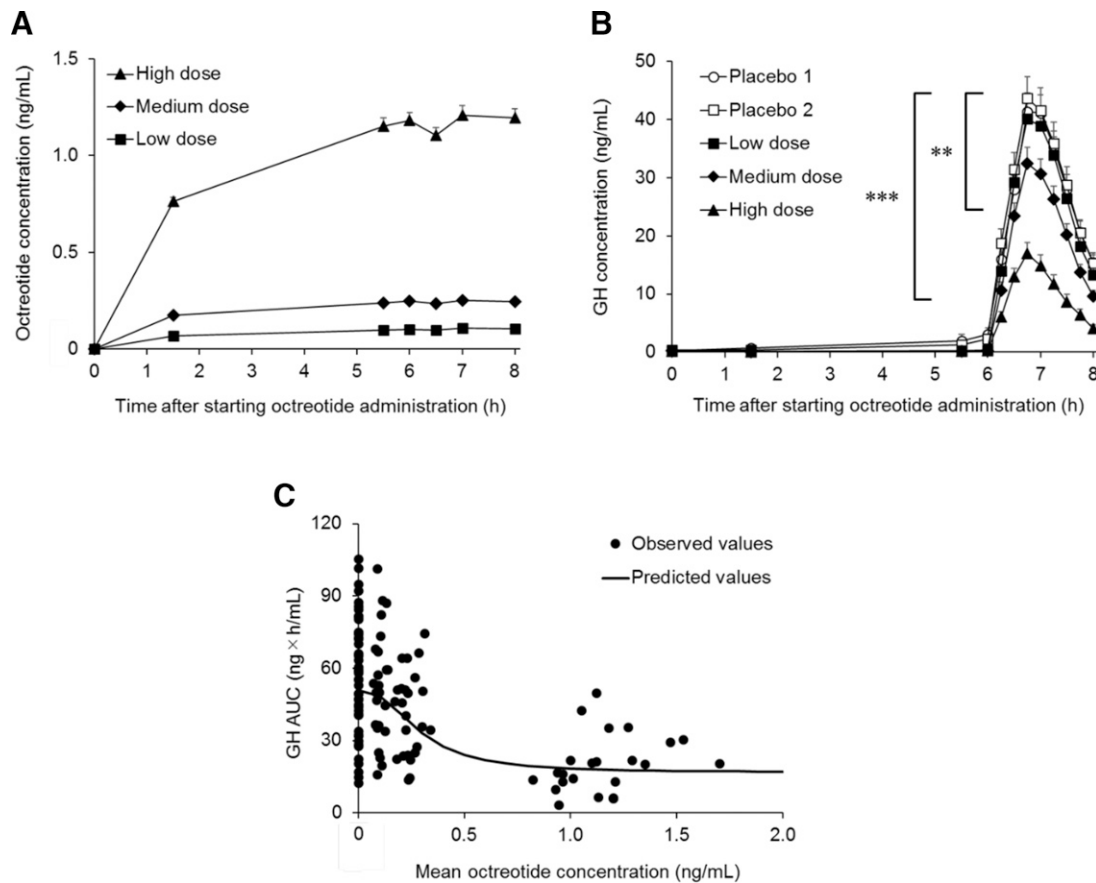


Fig. 2. Inhibitory effects of octreotide on GH secretion after combined administration of GHRH and arginine in humans. Healthy male participants received placebo or several doses of octreotide by intravenous infusion over 8 hours (low dose, 8.5 μg/200 ml; medium dose, 21 μg/200 ml; high dose, 92 μg/200 ml). Six hours after starting octreotide/placebo administration, the participants received an intravenous bolus of GHRH and 30-minute intravenous infusion of L-arginine. Plasma octreotide concentration (A) and serum GH concentration (B) were expressed as mean + S.E.M. ($n = 24$); one-way analysis of variance followed by Dunnett’s test was applied to comparison of GH AUC between the vehicle control group and test octreotide-treated groups; ** $P < 0.01$ and *** $P < 0.001$ vs. placebo controls. (C) Relationship between GH and octreotide concentrations.

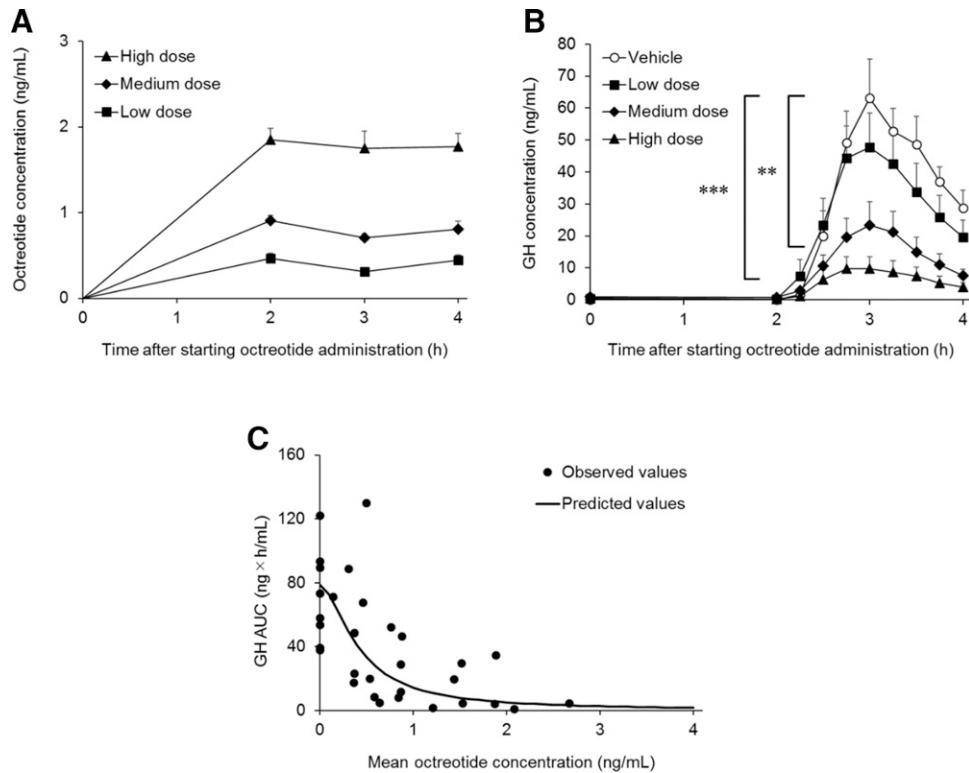


Fig. 3. Inhibitory effects of octreotide on GH secretion after combined administration of GHRH and arginine in monkeys. Male cynomolgus monkeys were administered vehicle or several doses of octreotide by intravenous infusion over 4 hours. The infusion rate varied between the first 2 hours and the subsequent 2 hours (low dose, $0.233 \rightarrow 0.133 \mu\text{g}/\text{kg}$ per hour; medium dose, $0.467 \rightarrow 0.27 \mu\text{g}/\text{kg}$ per hour; high dose: $0.7 \rightarrow 0.4 \mu\text{g}/\text{kg}$ per hour). An intravenous bolus of GHRH and 30-minute intravenous infusion of L-arginine were administered 2 hours after starting octreotide/vehicle administration. Plasma octreotide concentration (A) and plasma GH concentration (B) were expressed as mean + S.E.M. ($n = 8$); one-way analysis of variance followed by Dunnett's test was applied to comparison of GH AUC between the vehicle control group and test octreotide-treated groups; $**P < 0.01$ and $***P < 0.001$ vs. vehicle controls. (C) Relationship between GH and octreotide concentrations.

administration of GHRH and arginine was $71.0 \pm 10.4 \text{ ng} \times \text{h}/\text{ml}$, and the GH AUC was significantly inhibited in a dose-dependent manner after administration of low-, medium-, and high-dose octreotide of $58.4 \pm 13.9 \text{ ng} \times \text{h}/\text{ml}$, $P = 0.693$; $23.0 \pm 7.45 \text{ ng} \times \text{h}/\text{ml}$, $P < 0.01$; and $12.5 \pm 4.79 \text{ ng} \times \text{h}/\text{ml}$, $P < 0.001$, respectively (Fig. 3B). In the monkey E-R model, there was no significant decrease in OFV when E_{max} was estimated as a parameter, and this was therefore fixed at 1. The estimated EC_{50} was $0.416 \text{ ng}/\text{ml}$, and interindividual variability was incorporated (Fig. 3C).

In Vivo Study in Rats. The octreotide and GH concentrations and E-R relationship between GH and octreotide concentration after GHRH stimulation in rats are shown in Fig. 4. The plasma octreotide concentration 35 minutes after octreotide administration increased dose-proportionally ($0.0978 \pm 0.0223 \text{ ng}/\text{ml}$ at $0.32 \mu\text{g}/\text{kg}$, $0.229 \pm 0.0179 \text{ ng}/\text{ml}$ at $0.65 \mu\text{g}/\text{kg}$, $0.460 \pm 0.0225 \text{ ng}/\text{ml}$ at $1.3 \mu\text{g}/\text{kg}$, $0.828 \pm 0.0625 \text{ ng}/\text{ml}$ at $2.2 \mu\text{g}/\text{kg}$, and $3.33 \pm 0.210 \text{ ng}/\text{ml}$ at $6.5 \mu\text{g}/\text{kg}$ octreotide) (Fig. 4A). The plasma GH concentration 5 minutes after GHRH administration in the vehicle group was $1340 \pm 90.4 \text{ ng}/\text{ml}$. Octreotide significantly inhibited GHRH-induced GH secretion in a dose-dependent manner (plasma GH concentrations: $1030 \pm 105 \text{ ng}/\text{ml}$, $P = 0.0991$; $674 \pm 120 \text{ ng}/\text{ml}$, $P < 0.001$; $363 \pm 126 \text{ ng}/\text{ml}$, $P < 0.001$; $142 \pm 36.9 \text{ ng}/\text{ml}$, $P < 0.001$; and $24.6 \pm 3.72 \text{ ng}/\text{ml}$, $P < 0.001$ at 0.32 , 0.65 , 1.3 , 2.2 , and $6.5 \mu\text{g}/\text{kg}$ octreotide) (Fig. 4B). E_{max} was fixed at 1 in the rat E-R model, given that complete inhibition of GH prevented

E_{max} from converging. EC_{50} was estimated as $0.196 \text{ ng}/\text{ml}$ (Fig. 4C).

E-R in Rats, Monkeys, and Humans. The model-predicted E-R relationships in rats, monkeys, and humans are shown in Fig. 5, and the parameter estimates are given in Table 2. We referred to the FDA databases of new drug applications for the E-R relationship in patients with acromegaly, and the predicted results are shown in Fig. 5 (octreotide responder after subcutaneous administration; $EC_{50} = 0.2647 \text{ ng}/\text{ml}$, Hill = 1.5; FDA, 1998). For comparison, octreotide effect was calculated as a percentage of E_{max} normalized to 100% in each species. The relative standard errors (standard error as a percentage of estimate) for all fixed-effect parameters were $< 30\%$, which suggests that the model analyses were robust enough to compare the E-R relationships between species. There were good agreements of the E-R relationship between healthy participants and patients with acromegaly and between humans and animals.

Discussion

This study aimed to develop a novel platform for predicting drug response in patients with acromegaly by translational E-R evaluation of the GH-inhibitory effect of drugs in healthy participants and animals with stimulated GH levels. This study provides the first evidence demonstrating consistent E-R

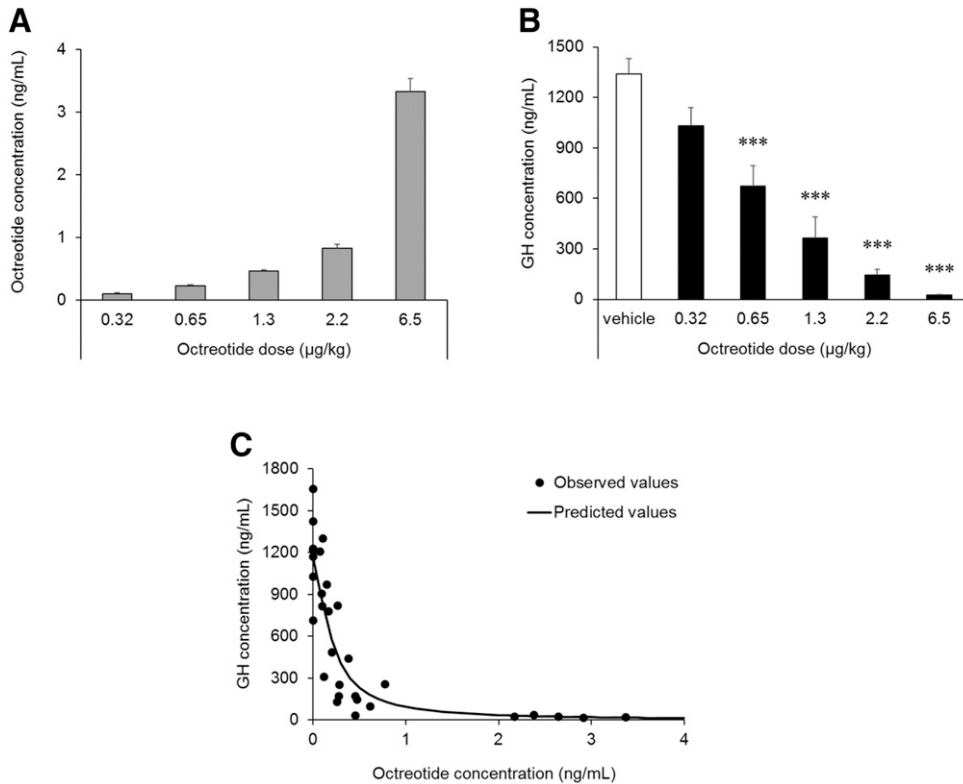


Fig. 4. Inhibitory effects of octreotide on GH secretion after GHRH administration in rats. Male Crl:CD (Sprague-Dawley) IGS rats were administered vehicle or several doses of octreotide subcutaneously, which were followed by GHRH injection 30 minutes after octreotide/vehicle administration. Blood samples were collected 5 minutes after GHRH administration. Plasma octreotide concentration (A) and plasma GH concentration (B) were expressed as mean + S.E.M. ($n = 5$); one-way analysis of variance followed by Dunnett's test was applied to comparison of GH between the vehicle control group and test octreotide-treated groups; *** $P < 0.001$ vs. vehicle controls. (C) Relationship between GH and octreotide concentrations.

relationships of octreotide as a representative SSA among animals, healthy participants, and patients with acromegaly.

There are various ways of stimulating GH levels in healthy participants, but peak GH levels are not very high (approximately 10–20 ng/ml) after stimulation with a single agent, such as GHRH, arginine, or GH-releasing peptides (Iranmanesh et al., 2004; Berg et al., 2009). To mitigate the effects of diurnal variation in irregular-pulsed GH secretion, GH can be stimulated by the combined administration of GHRH and arginine, which results in the secretion of higher levels of GH (Tuvia

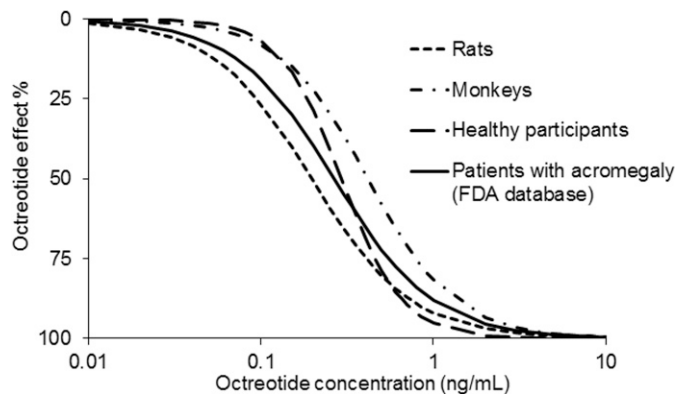


Fig. 5. Exposure response of the growth hormone-inhibitory effect of octreotide after growth hormone-releasing hormone (+ arginine)-stimulation in rats, monkeys, and humans. Patients with acromegaly derived from the FDA database were also included (FDA, 1998). Octreotide effect was calculated as a percentage of E_{max} normalized to 100% in each species. FDA, Food and Drug Administration.

et al., 2012). To obtain an accurate E-R relationship, we administered octreotide by intravenous infusion to maintain a constant concentration during GH stimulation. Assuming that this system could be used to evaluate novel oral compounds in the future, we evaluated GH and octreotide concentrations 6–8 hours after the start of octreotide administration, when the pharmacokinetics of most of oral drugs would reach the elimination phase. In previous GH stimulation studies in humans, although GH stimulation was performed 2–3 hours after administration of SSA (Tuvia et al., 2012; Golor et al., 2012), it may correspond to the absorption phase of oral compounds and, thus, would not be appropriate for pharmacological evaluation. We also used intermittent administration of octreotide and GHRH + arginine in a crossover design to obtain the E-R relationship for individual healthy participants. The main concern prior to conducting this study was a lack of reproducibility in the stimulated GH levels because of desensitization by octreotide (Escorsell et al., 2001), which is considered to result from receptor phosphorylation and internalization (Hipkin et al., 1997). However, the results showed that GHRH + arginine-stimulated secretion of GH after placebo administration in the fixed treatment period and in a randomized treatment period was comparable, which confirmed the reproducibility of the response with no carryover effect with a 7-day washout period between repeated administrations of octreotide acetate. This system was therefore considered suitable for evaluating the pharmacodynamics of SSAs. Additionally, the administration of different doses of octreotide allowed us to assess the dose-dependent inhibition of GH. Unlike in monkeys and rats, GH

TABLE 2

Parameter estimates of the exposure–response model in rats, monkeys, and humans. E_{\max} in human was estimated using a logistic function ($E_{\max} = e^{\text{logit}} / (1 + e^{\text{logit}})$).

Parameter	Estimates (RSE%)		
	Rat	Monkey	Human
E_{\max}	1 fixed	1 fixed	0.670
Logit for E_{\max}	—	—	0.707 (16.3)
EC_{50} (ng/ml)	0.196 (22.7)	0.416 (29.3)	0.292 (7.60)
Hill	1.50 (7.33)	1.71 (26.3)	2.44 (8.44)
E_0 (ng/ml ^a or ng×h/ml ^b)	1170 (8.47) ^a	78.9 (11.2) ^b	50.7 (9.90) ^b
ω^2 Logit for E_{\max}	—	—	0.520 (51.3)
$\omega^2 EC_{50}$	—	0.693 (48.5)	—
$\omega^2 E_0$	—	—	0.219 (30.0)
σ^2 Proportional	0.195 (28.3)	0.128 (29.0)	0.0260 (14.0)

ω^2 , interindividual variance estimate for each parameter; σ^2 , proportional residual error variance; RSE%, relative standard error (standard error as a percentage of estimate).

stimulation in humans was not completely inhibited by even the highest dose of octreotide acetate, with an estimated E_{\max} of 67%. The mean plasma octreotide concentration at the highest dose was 1.15 ng/ml, which was comparable to the maximal effective concentration of octreotide in patients with acromegaly (FDA, 1998). Similar results have been reported in clinical studies with oral octreotide (Tuvia et al., 2012). Although inhibition of GH secretion from human pituitary cells is mediated by both sst2 and sst5 subtypes, octreotide has high affinity for sst2 and lower affinity for sst5 (Ben-Shlomo and Melmed, 2008). This selective binding affinity of octreotide for sst2 may be responsible for the incomplete and individually variable E_{\max} values for GH inhibition. Activation of both sst2 and sst5 induces a functional association of receptor subtypes, which results in synergistic GH suppression (Ren et al., 2003). In a clinical study, octreotide had no effect in some patients with acromegaly with low sst2 expression, whereas pasireotide had a superior inhibitory effect on GH secretion in these patients by acting on both sst2 and sst5 (van der Hoek et al., 2004).

The monkey study was designed as a preclinical study to mimic the human study. Studies of SSAs in monkeys are currently lacking, although monkeys are used as nonrodent models to assess the efficacy, safety, and pharmacokinetics of drugs at the preclinical stage. Indeed, the inhibitory effect of pasireotide on the GH/IGF-1 axis have been tested in monkeys (Weckbecker et al., 2002). The current animal studies were conducted under anesthesia because GH levels are disturbed when the animals become excited. The duration of octreotide infusion was determined to be 4 hours based on the duration of anesthesia and the level of sedation of the monkeys. We also conducted a study in rats, representing a more convenient system for screening novel candidate drugs. The behavior of GH in rats was different from that in humans and monkeys. Initially, we tried intravenous infusion as a preliminary study in rats. When octreotide was administered by continuous intravenous infusion for 8 hours, the effect of octreotide was reduced by approximately 15% in a time-dependent manner (unpublished data). Such desensitization has not been observed in patients with acromegaly (Chanson et al., 1993) or in healthy male subjects (Beglinger et al., 2012). Because of concerns about misinterpretation of the octreotide effects across species by desensitization, we minimized the exposure time of octreotide in rats. Octreotide has previously been reported to be well distributed in systemic organs in rats 30 minutes after administration (Lemaire et al., 1989), which was considered appropriate for evaluating the response. Unlike

humans and monkeys, arginine was not administered to the rats because sufficient GH was secreted after administration of GHRH alone. GH peaks in only 5 minutes and eliminates within 30 minutes when rats are stimulated with GHRH (Tulipano et al., 2002). Because peak GH and GH AUC are strongly correlated, we thought it possible to evaluate the effect of octreotide at peak GH in rats. Practically, octreotide was administered by subcutaneous bolus, and GH was evaluated 5 minutes after GHRH administration for minimal blood-sampling design. These animal studies also revealed a clear E-R relationship between plasma concentrations of octreotide and GH. Unlike other parts of the brain, the pituitary gland, where octreotide mainly acts, lacks tight junctions between endothelial cells (Wilhelm et al., 2016). Octreotide is thus easily distributed from the blood, and concentrations around the target are closely correlated with plasma concentrations. *In vivo* plasma concentration-based EC_{50} values were consistent across species, which was in line with the lack of species differences in sst2 agonistic activity of octreotide *in vitro*.

The pharmacokinetic/pharmacodynamic analysis of GH suppression in patients with acromegaly treated with SSAs showed a Hill coefficient, representing the slope of the concentration-response relationship, above 1 for all SSAs (Ma et al., 2005; Garrido et al., 2012). This indicates that the drug effect increased steeply with increasing SSA blood concentration, which suggests that the dosage should be selected more strictly. Interestingly, our studies reflected not only the EC_{50} but also the steep E-R relationship in patients with acromegaly. These results indicated that stimulated GH studies allowed simulation of the dosage and therapeutic effects against acromegaly based on early research and development stages. Our study was conducted exclusively for males in all species tested since the GH response to stimulation by GHRH + arginine is sex-dependent (Markkanen et al., 2017). Our studies reflected the E-R relationship of male and female patients with acromegaly, but an important limitation is the limited background of subjects in our studies. As noted above, the demonstrated E-R relationship was primarily based on sst2 agonistic activity, and attention should thus be paid to sst selectivity when applying this system to other compounds. Additionally, when predicting clinical effects based on animal studies, it is important to determine the existence of species differences in sst agonistic activity using *in vitro* studies.

In conclusion, the E-R relationship between octreotide and GHRH + arginine-stimulated GH in healthy participants reflected the relationship in patients with acromegaly.

Additionally, the response in humans could be predicted by conducting relevant animal studies. These findings will help in the development of a novel platform to simulate the dosage and therapeutic effects of drugs targeting acromegaly and will facilitate the research and development of novel therapeutic agents with similar modes of action.

Authorship Contributions

Participated in research design: Iida, Komagata, Kitagawa, Shinozaki, Seki, Bruce, Ohno.

Conducted experiments: Komagata, Tanaka, Nagasawa, Nishio, Shono.

Performed data analysis: Iida.

Wrote or contributed to the writing of the manuscript: Iida, Ogawara, Ohno.

References

- Beglinger C, Hu K, Wang Y, Bouillaud E, Darstein C, Wang Y, and Mohideen P (2012) Multiple once-daily subcutaneous doses of pasireotide were well tolerated in healthy male volunteers: a randomized, double-blind, placebo-controlled, cross-over, Phase I study. *Endocrine* **42**:366–374.
- Ben-Shlomo A and Melmed S (2008) Somatostatin agonists for treatment of acromegaly. *Mol Cell Endocrinol* **286**:192–198.
- Berg CA, Pokrajac A, Bidlingmaier M, Strasburger CJ, Shalet SM, and Trainer PJ (2009) Use of a GH receptor antagonist (GHRA) to explore the relationship between GH and IGF-I in adults with severe GH deficiency (GHD). *Clin Endocrinol (Oxf)* **70**:439–445.
- Chanson P and Salenave S (2008) Acromegaly. *Orphanet J Rare Dis* **3**:17.
- Chanson P, Timsit J, and Harris AG (1993) Clinical pharmacokinetics of octreotide. Therapeutic applications in patients with pituitary tumours. *Clin Pharmacokinet* **25**:375–391.
- Dimaraki EV, Jaffe CA, Bowers CY, Marbach P, and Barkan AL (2003) Pulsatile and nocturnal growth hormone secretions in men do not require periodic declines of somatostatin. *Am J Physiol Endocrinol Metab* **285**:E163–E170.
- Dimaraki EV, Jaffe CA, Demott-Friberg R, Russell-Aulet M, Bowers CY, Marbach P, and Barkan AL (2001) Generation of growth hormone pulsatility in women: evidence against somatostatin withdrawal as pulse initiator. *Am J Physiol Endocrinol Metab* **280**:E489–E495.
- Doi N, Hirotsu C, Ukai K, Shimada O, Okuno T, Kurasaki S, Kiyofuji T, Ikegami R, Futamata M, Nakagawa T, et al. (2004) Pharmacological characteristics of KP-102 (GHRP-2), a potent growth hormone-releasing peptide. *Arzneimittelforschung* **54**:857–867.
- Escorsell A, Bandi JC, Andreu V, Moitinho E, García-Pagán JC, Bosch J, and Rodés J (2001) Desensitization to the effects of intravenous octreotide in cirrhotic patients with portal hypertension. *Gastroenterology* **120**:161–169.
- Food and Drug Administration (2003) Guidance for industry. Exposure-response relationships — study design, data analysis, and regulatory applications. <https://www.fda.gov/media/71277/download>.
- Food and Drug Administration (1998) Drug approvals and databases for Sandostatin lar depot (octreotide acetate) injection. Application No.: 021-008. Clinical pharmacology biopharmaceutics review(s). https://www.accessdata.fda.gov/drugsatfda_docs/nda/98/021008a_clinphrm.pdf.
- Fleseriu M, Biller BMK, Freda PU, Gadelha MR, Giustina A, Katznelson L, Molitch ME, Samson SL, Strasburger CJ, van der Lely AJ, et al. (2021) A Pituitary Society update to acromegaly management guidelines. *Pituitary* **24**:1–13.
- Gadelha MR, Bronstein MD, Brue T, Coculescu M, Fleseriu M, Guitelman M, Pronin V, Raverot G, Shimon I, Lievre KK, et al.; Pasireotide C2402 Study Group (2014) Pasireotide versus continued treatment with octreotide or lanreotide in patients with inadequately controlled acromegaly (PAOLA): a randomised, phase 3 trial. *Lancet Diabetes Endocrinol* **2**:875–884.
- Gadelha MR, Kasuki L, Lim DST, and Fleseriu M (2019) Systemic complications of acromegaly and the impact of the current treatment landscape: an update. *Endocr Rev* **40**:268–332.
- Garrido MJ, Cendrós JM, Ramis J, Peraire C, Obach R, and Trocóniz IF (2012) Pharmacodynamic modeling of the effects of lanreotide Autogel on growth hormone and insulin-like growth factor I. *J Clin Pharmacol* **52**:487–498.
- Giustina A, Barkhoudarian G, Beckers A, Ben-Shlomo A, Biermasz N, Biller B, Boguszewski C, Bolanowski M, Bollerslev J, Bonert V, et al. (2020) Multidisciplinary management of acromegaly: a consensus. *Rev Endocr Metab Disord* **21**:667–678.
- Golor G, Hu K, Ruffin M, Buchelt A, Bouillaud E, Wang Y, and Maldonado M (2012) A first-in-man study to evaluate the safety, tolerability, and pharmacokinetics of pasireotide (SOM230), a multireceptor-targeted somatostatin analog, in healthy volunteers. *Drug Des Devel Ther* **6**:71–79.
- Hipkin RW, Friedman J, Clark RB, Eppler CM, and Schonbrunn A (1997) Agonist-induced desensitization, internalization, and phosphorylation of the sst2A somatostatin receptor. *J Biol Chem* **272**:13869–13876.
- Ho KKY; 2007 GH Deficiency Consensus Workshop Participants (2007) Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. *Eur J Endocrinol* **157**:695–700.
- Holdaway IM, Rajasoorya RC, and Gamble GD (2004) Factors influencing mortality in acromegaly. *J Clin Endocrinol Metab* **89**:667–674.
- Iranmanesh A, Bowers CY, and Veldhuis JD (2004) Activation of somatostatin-receptor subtype-2/-5 suppresses the mass, frequency, and irregularity of growth hormone (GH)-releasing peptide-2-stimulated GH secretion in men. *J Clin Endocrinol Metab* **89**:4581–4587.
- Lemaire M, Azria M, Dannecker R, Marbach P, Schweitzer A, and Maurer G (1989) Disposition of Sandostatin, a new synthetic somatostatin analogue, in rats. *Drug Metab Dispos* **17**:699–703.
- Ma P, Wang Y, van der Hoek J, Nedelman J, Schran H, Tran LL, and Lamberts SWJ (2005) Pharmacokinetic-pharmacodynamic comparison of a novel multiligand somatostatin analog, SOM230, with octreotide in patients with acromegaly. *Clin Pharmacol Ther* **78**:69–80.
- Manolopoulos J, Alami Y, Petersenn S, Schopohl J, Wu Z, Strasburger CJ, and Bidlingmaier M (2012) Automated 22-kD growth hormone-specific assay without interference from Pegvisomant. *Clin Chem* **58**:1446–1456.
- Markkanen HM, Pekkarinen T, Hämäläinen E, Välimäki MJ, Alftan H, and Stenman UH (2017) Gender has to be taken into account in diagnosing adult growth hormone deficiency by the GHRH plus arginine test. *Growth Horm IGF Res* **35**:52–56.
- Melmed S (2009) Acromegaly pathogenesis and treatment. *J Clin Invest* **119**:3189–3202.
- Pharmaceuticals and Medical Devices Agency (2020) Guideline for exposure-response analysis of drugs. <https://www.pmda.go.jp/files/000235382.pdf>.
- Ren SG, Taylor J, Dong J, Yu R, Culler MD, and Melmed S (2003) Functional association of somatostatin receptor subtypes 2 and 5 in inhibiting human growth hormone secretion. *J Clin Endocrinol Metab* **88**:4239–4245.
- Schmid HA and Schoeffter P (2004) Functional activity of the multiligand analog SOM230 at human recombinant somatostatin receptor subtypes supports its usefulness in neuroendocrine tumors. *Neuroendocrinology* **80** (Suppl 1):47–50.
- Strasburger CJ, Karavitaki N, Störmann S, Trainer PJ, Kreitschmann-Andermahr I, Droste M, Korbonits M, Feldmann B, Zopf K, Sanderson VF, et al. (2016) Patient-reported outcomes of parenteral somatostatin analogue injections in 195 patients with acromegaly. *Eur J Endocrinol* **174**:355–362.
- Tiberg F, Roberts J, Cervin C, Johnsson M, Sarp S, Tripathi AP, and Linden M (2015) Octreotide s.c. depot provides sustained octreotide bioavailability and similar IGF-1 suppression to octreotide LAR in healthy volunteers. *Br J Clin Pharmacol* **80**:460–472.
- Tulipano G, Soldi D, Bagnasco M, Culler MD, Taylor JE, Cocchi D, and Giustina A (2002) Characterization of new selective somatostatin receptor subtype-2 (sst2) antagonists, BIM-23627 and BIM-23454. Effects of BIM-23627 on GH release in anesthetized male rats after short-term high-dose dexamethasone treatment. *Endocrinology* **143**:1218–1224.
- Tuvia S, Atsmon J, Teichman SL, Katz S, Salama P, Pelled D, Landau I, Karmeli I, Bidlingmaier M, Strasburger CJ, et al. (2012) Oral octreotide absorption in human subjects: comparable pharmacokinetics to parenteral octreotide and effective growth hormone suppression. *J Clin Endocrinol Metab* **97**:2362–2369.
- van der Hoek J, de Herder WW, Feelders RA, van der Lely AJ, Uitterlinden P, Boerlin V, Bruns C, Poon KW, Lewis I, Weckbecker G, et al. (2004) A single-dose comparison of the acute effects between the new somatostatin analog SOM230 and octreotide in acromegalic patients. *J Clin Endocrinol Metab* **89**:638–645.
- Weckbecker G, Briner U, Lewis I, and Bruns C (2002) SOM230: a new somatostatin peptidomimetic with potent inhibitory effects on the growth hormone/insulin-like growth factor-I axis in rats, primates, and dogs. *Endocrinology* **143**:4123–4130.
- Wilhelm I, Nyúl-Tóth Á, Suciú M, Hermenean A, and Krizbai IA (2016) Heterogeneity of the blood-brain barrier. *Tissue Barriers* **4**:e1143544.

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