

Growth Hormone Bioavailability, Insulin-Like Growth Factor-I and IGF-Binding-Protein-3 Release in Japanese and Caucasian Subjects

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Abstract

Context: The pharmacokinetics (PK) of the recombinant human GH (rhGH) is poorly documented for the Japanese adult population, and a study comparing the PK, insulin-like growth factor-I (IGF-I) and IGF-binding-protein-3 (IGFBP-3) release between Japanese and Caucasian subjects after rhGH administration has previously not been reported.

Objective: To compare the profiles of serum GH concentrations and the IGF-I and IGFBP-3 responses after administration of identical doses of rhGH to healthy Japanese and Caucasian subjects.

Design and Setting: A randomised, double-blind, placebo-controlled, parallel-group study.

Participants and Intervention: A total of 80 healthy male subjects (40 Japanese and 40 Caucasians) completed the study. A single dose of rhGH or placebo was administered subcutaneously, and blood samples were drawn up to 24 hours post-administration.

Main Outcome Measures: Standard PK parameters, including the area under the GH concentration–time curve from 0 to 24 hours ($AUC_{(0-24h)}$) and maximum GH concentration from 0 to 24 hours (C_{max}). The IGF-I and IGFBP-3 levels were measured at various timepoints during the sampling period.

Results: The bioavailabilities for Japanese and Caucasian subjects in terms of $AUC_{(0-24 h)}$ and C_{max} were considered equivalent. The time to maximum GH concentration from 0–24 hours (t_{max}) was not statistically different for Japanese and Caucasian subjects. No differences in IGF-I or IGFBP-3 levels were observed.

Conclusion: The bioavailabilities of rhGH for Japanese and Caucasian subjects are considered equivalent. Basal circulating IGF-I and IGFBP-3 levels and the release of IGF-I and IGFBP-3 after administration of rhGH were similar between the two ethnic populations.

Keywords: Growth hormone; IGF-I; IGFBP-3

Abbreviations: AUC: Area under the Curve; BMI: Body Mass Index; C_{max} : Maximum Concentration; CI: Confidence Interval; ELISA: Enzyme-Linked Immunosorbent Assay; FDA: Food and Drug Administration; PD: Pharmacodynamics; PK: Pharmacokinetics; rhGH: Recombinant Human Growth Hormone; $t_{1/2}$: Terminal Half-Life; t_{max} : Time to Maximum Concentration

Introduction

Recombinant human GH (rhGH) is the standard treatment for severe growth retardation resulting from GH deficiency or insufficiency in children, and other conditions such as Turner's syndrome, small for gestational age, and Noonan syndrome. The efficacy of GH replacement in GH-deficient adults is now well established [1-3]. The major biological effect of rhGH is stimulation of skeletal and somatic growth, but rhGH is also known to exert numerous effects on body composition, carbohydrate, lipid and protein metabolism [4].

It could be speculated whether ethnic differences in the pharmacokinetics (PK) and pharmacodynamics (PD) of rhGH exists as a result of general differences in metabolism, excretion and secretion. If this is the case, it has implications for the bridging of results obtained during clinical studies conducted in different races. The PK of rhGH within the Japanese adult population is very poorly documented, and a comparison of the PK profiles and rhGH-mediated IGF-I release between Japanese and Caucasian subjects after rhGH administration has previously not been reported. The present study therefore investigated standard PK parameters after single administrations of rhGH to healthy male Japanese and the Caucasian counterpart. As the influence

of the endogenous production may compromise such comparison, the intrinsic release of GH was suppressed by somatostatin infusion prior to administration of rhGH [5], a method that has successfully been employed in rhGH bioequivalence studies [6,7]. To our knowledge, this is the first reported study comparing the PK and rhGH-mediated release of IGF-I and IGFBP-3 after GH administration in Japanese and Caucasian subjects.

Subjects and Methods

Subjects

The study was conducted in a single clinical trial centre (Radiant Research, Honolulu, Hawaii, USA). Healthy, non-smoking Japanese and Caucasian male subjects aged ≥ 20 to ≤ 50 , with a body mass index (BMI) of ≥ 17 to ≤ 30 kg/m², were eligible for the study, and key exclusion criteria were regular use of prescription medicine, a history of alcohol or drug abuse, diabetes or a relative with first-degree diabetes

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mellitus, substantial intake of caffeine-containing beverages (equal to > 8 daily cups of coffee), night shift labour, and positive test for hepatitis B or C, or HIV. The protocol was approved by the Washington Independent Review Board (WIRB) and by the U.S. Food and Drug Administration (FDA) prior to study initiation, and the study was performed in accordance with the Declaration of Helsinki. The subjects were informed about all its aspects and signed informed consent forms before any study-related procedure.

Study design

The study was a randomised, double-blind, placebo-controlled, parallel-group, phase 1 clinical trial, with the primary objective to investigate the PK of rhGH in healthy Japanese and Caucasian subjects. The secondary objectives were to investigate IGF-I and IGFBP-3 levels, and to identify isolated somatostatin-related adverse events and confirm that the somatostatin method functioned as expected.

Subjects were randomly assigned to either placebo or active treatment. Placebo groups were included with the purpose of enabling evaluation of the somatostatin treatment. Placebo was liquid solvent for rhGH but without the active compound (i.e. rhGH). The study was double-blind primarily to blind the person that performed the sc injections and avoid potential administration bias related to injections. Parallel group design was needed to compare Japanese and Caucasian subjects.

Study procedures and assays

Subjects visited the clinic three times: at a screening visit, a visit 4–21 days after the screening and at a post-study visit. Somatostatin was infused from 2 hours before the administration of a single subcutaneous dose of 5 mg rhGH at 0 hours, until the last plasma sampling point (24 hours post-dose). The somatostatin dose was 120 µg/hour from –2 hours to 0 hours, and was then changed to 50 µg/hour for the remaining 24-hour infusion period. Study products administered were the rhGH Norditropin® (Novo Nordisk A/S, Denmark), and the somatostatin Octreotide® (Novartis, U.S.). Subjects fasted overnight from 22.00 hours (water was allowed). A food supplement beverage was served approximately 2 hours before administration of rhGH in the thigh, and immediately before start of the somatostatin infusion (time –2 hours). Food supplement beverages were offered every second hour during the somatostatin infusion in order to prevent hypoglycaemia.

Venous blood samples were drawn immediately before intake of the first food supplement and start of the somatostatin infusion (–2 hours), and –20 minutes and –10 minutes before administration of rhGH (time 0 hours). Subsequent sampling was performed 15 minutes following rhGH administration, every half-hour from 30 minutes to 6 hours, and then every hour from 7–24 hours following administration; in total 35 samplings in accordance with previous studies [7,8]. The blood glucose was measured at each blood sampling, and the total amount of blood drawn from each subject during the study did not exceed 300 ml, being less than the volume of a blood donation. The serum concentrations of GH were analysed using a commercially available enzyme-linked immunosorbent assay with a lower limit of quantification being 0.1 µg/L (ELISA) (Diagnostic System Laboratories Inc., Texas, USA). Serum samples collected at –2, 0, 12 and 24 hours were analysed for IGF-I and IGFBP-3 levels, using commercially available ELISAs (Diagnostic System Laboratories Inc., Texas, USA). The primary endpoints of the study were $AUC_{(0-24h)}$ and C_{max} . Secondary endpoints were $AUC_{(0-\infty)}$, t_{max} and $t_{1/2}$.

Statistical analysis

The primary objective of the PK analysis was to compare the PK for healthy Japanese and Caucasian subjects, by estimating the relative bioavailability in terms of $AUC_{(0-24h)}$ and C_{max} . The PK was considered equivalent if the mean for one population receiving active treatment was within 80%–125% of the mean of the other population receiving active treatment. The null hypothesis was that the PK of active treatment was non-equivalent between the two populations. The hypothesis was tested by calculating a 90% confidence interval for the ratio between means for the two populations using an analysis of variance (ANOVA) model, with race as fixed effect. The analysis was performed on the log-transformed values of $AUC_{(0-24h)}$ and C_{max} . Additional analyses were performed for $AUC_{(0-24h)}$ adjusting for BMI and dose per weight, respectively. Analysis of covariance (ANCOVA) was applied in two separate analyses for the log-transformed $AUC_{(0-24h)}$ with log-transformed BMI and log-transformed dose (adjusted for weight) as covariates, respectively, and the estimated mean ratio between Japanese and Caucasians with corresponding 90% CI was calculated in order to assess the equivalence. The t_{max} was analysed non-parametrically (Jonckheere-Terpstra test, two-sided). The $AUC_{(0-\infty)}$ and $t_{1/2}$ were summarised. IGF-I and IGFBP-3 data was analysed using descriptive statistics in accordance with the study protocol.

Somatostatin-related adverse events were analysed for differences between the two populations using the Fisher's exact test. The events were identified based on the adverse events observed during the 2-hour somatostatin infusion prior to the administration of rhGH treatment bolus, the adverse events observed during the 24-hour somatostatin infusion period after the placebo treatment as compared to active treatment, and the known side-effects of somatostatin.

The SAS system version 6.12 for Windows, or the corresponding version for UNIX, was used for all statistical analyses.

Results

Subject demography and accountability

A total of 86 healthy male subjects (42 Japanese and 44 Caucasians) were randomised into the study (Figure 1). The mean (standard deviation; SD) age and BMI value for Japanese was 36.1 (8.7) years and 25.0 (2.8) kg/m², respectively, and for Caucasians 33.5 (8.8) years and 23.8 (2.2) kg/m², respectively.

Pharmacokinetics

Baseline characteristics: Of the 80 subjects completing the study, 75 subjects were included in the PK analysis population, which was composed of all subjects abiding by the study protocol. The five subjects were excluded as they met exclusion criteria: three subjects had worked night shifts, one subject had a first-degree relative with diabetes mellitus, and one subject suffered from depression that required regular medication. In the PK analysis population, 67 (89%) were treated with rhGH (32 Japanese and 35 Caucasians) and 8 with placebo (3 Japanese and 5 Caucasians). The somatostatin infusion suppressed the endogenous GH secretion successfully, as verified by GH concentrations below the limit of quantification (0.1 µg/L) at timepoints prior to rhGH administration, and throughout the sampling period in subjects receiving placebo (data not shown). Thus, the contribution of endogenous GH measured over the sampling period was assumed to be negligible.

Primary endpoints: $AUC_{(0-24h)}$ and C_{max} The PK profiles obtained for the rhGH-exposed subjects within the two study populations were

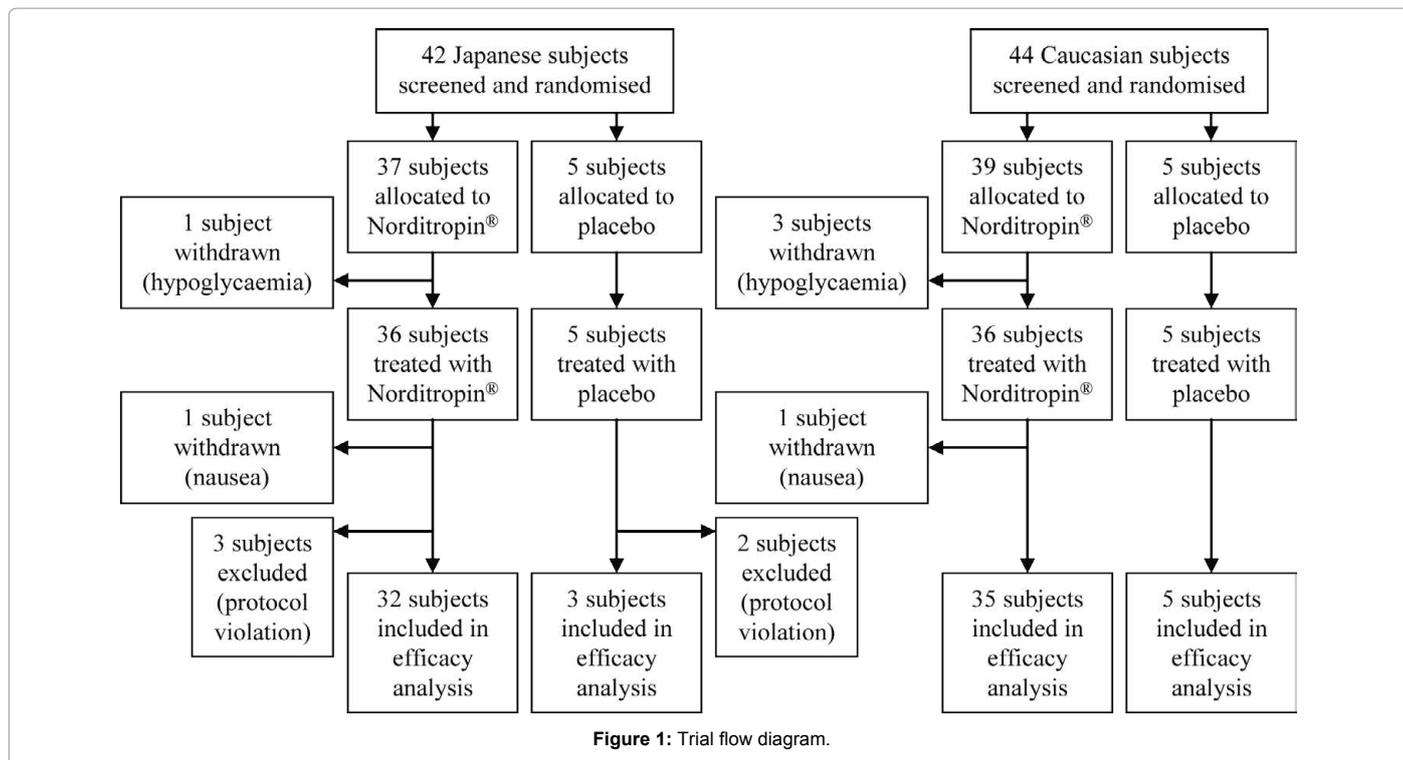


Figure 1: Trial flow diagram.

similar, with GH concentrations reaching maximum around 4–5 hours post-dose, and approaching 0 at 24 hours (Figure 2). The concentrations of GH in subjects receiving placebo were all below the lower limit of quantification (0.1 µg/L; data not shown). The mean $AUC_{(0-24\text{ h})}$ values were 253.0 and 242.5 µg × h/L for Japanese and Caucasian subjects, respectively (Table 1). The estimated mean ratio for the two populations was 1.039 (90% CI: 0.94–1.14; Table 1). The analysis was repeated for a second population composed of all randomised subjects who had started the somatostatin infusion (n=72). The estimated mean ratio was 1.034 (90% CI: 0.94–1.14). Further, statistical analyses on the original population (n=67) were performed, with adjustments for BMI and dose per weight. With log BMI as covariate, the estimated mean ratio was 1.139 and the 90% CI ranged from 1.049–1.236. With log dose (adjusted for weight) as covariate, the estimated ratio was 0.997 and the 90% CI ranged from 0.92–1.08. Hence, PK equivalence was indicated in both cases.

The mean C_{max} values were 24.5 and 22.6 µg/L for Japanese and Caucasian subjects, respectively, and the estimated mean ratio for the two populations was 1.045 (90% CI: 0.91–1.21; Table 1) and thus indicating equivalence. The analysis was repeated for the population that had started the somatostatin infusion (n=72), and as the estimated mean ratio was 1.00 (90% CI: 0.87–1.15), equivalence was demonstrated also for this analysis set.

Secondary endpoints: t_{max} , $AUC_{(0-\infty)}$, and $t_{1/2}$. The maximum GH concentrations (t_{max}) were obtained around 4–5 hours post-dose (Table 1), with no statistically significant difference between the two populations (Jonckheere-Terpstra test; $p=0.266$). The median elimination half-life values were also similar for both groups, with 3.8 and 3.2 hours for the Japanese and Caucasian population, respectively (Table 1). As the GH concentrations all approached 0 at 24 hours, the $AUC_{(0-\infty)}$ values differed only slightly from the $AUC_{(0-24\text{ hrs})}$ values (Figure 2; Table 1). Taken together, these results serve as further support for PK equivalence between the two populations investigated.

rhGH-induced release of IGF-I and IGFBP-3

No apparent differences between Japanese and Caucasian subjects in the levels of the basal circulating IGF-I or IGFBP-3 were observed. As expected, an overall, steady increase in IGF-I during the 24-hour sampling period following rhGH injection was observed in both populations (Figure 3a), as well as a similar increase for IGFBP-3, although less evident (Figure 3b). No substantial post-dose differences in IGF-I levels were observed when the two populations were compared. For Japanese subjects, the mean changes from 0 hours in IGF-I levels were 145.6 ng/ml (SD: 71.9 ng/ml) at 12 hours (range: -24.2 to 323.8 ng/ml) and 249.3 ng/ml (SD: 97.7 ng/ml) at 24 hours (range: 51.0 to 452.2 ng/ml). For Caucasian subjects, the mean changes from 0 hours in IGF-I levels were 92.0 ng/ml (SD: 89.1 ng/ml) at 12 hours (range: -180.0 to 251.4 ng/ml) and 218.0 ng/ml (SD: 110.9 ng/ml) at 24 hours (range: -91.3 to 385.2 ng/ml). No changes in IGF-I levels were observed within the group receiving placebo (data not shown). No post-dose differences in IGFBP-3 levels were observed when the two populations were compared, and no changes in IGFBP-3 levels were observed within the group receiving placebo (data not shown).

Safety: The bolus injection of 5 mg of rhGH was well tolerated and displayed similar safety profiles in the Japanese and Caucasian subjects. A total of 27 subjects experienced adverse events in the 2-hour period prior to treatment with rhGH or placebo (Japanese: 14 subjects (33%) reported 24 adverse events; Caucasian: 13 subjects (30%) reported 26 adverse events). The majority of the adverse events reported were injection-site reactions and somatostatin-related events (gastro-intestinal disorders and hypoglycaemia). Four subjects withdrew due to hypoglycaemic events before the administration of rhGH, between 35–47 minutes after initiation of the somatostatin infusion. All four hypoglycaemic episodes were classified as serious, and the somatostatin infusion was discontinued and glucose was administered.

A similar number of Japanese and Caucasian subjects experienced

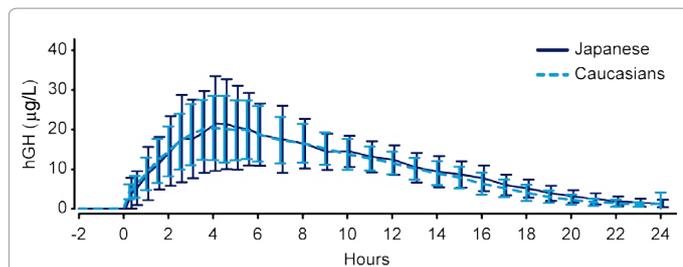


Figure 2: Mean (± 1 SD) serum GH concentrations in Japanese and Caucasian subjects after a single subcutaneous injection of rhGH, peaking at around 4–5 hours post-dose.

Population		AUC _(0–24 h) ($\mu\text{g} \times \text{h/L}$)	C _{max(0–24 h)} ($\mu\text{g/L}$)	AUC _(0–∞) ($\mu\text{g} \times \text{h/L}$)	t _{max} (hours)	t _{1/2} (hours)
Japanese (N=32)	Mean (SD);	253.0 (64.3)	24.5 (11.2)	262.9 (62.9)	5.3*	3.8*
	Range	147.5–387.2	12.1–57.4	157.8–391.6	2.6–14.1	1.5–10.7
Caucasians (N=35)	Mean (SD);	242.5 (58.7)	22.6 (8.3)	247.2 (57.6)	4.6*	3.2*
	Range	166.4–398.3	14.4–54.2	169.5–399.2	3.1–12.1	2.1–6.8
	Ratio**	1.039	1.045	n.d.	n.d.	n.d.
	90% CI	0.943–1.143	0.906–1.206	n.d.	n.d.	n.d.

n.d., not determined, *median, **estimated mean ratio between Japanese and Caucasians

Table 1: Pharmacokinetics in Japanese and Caucasian Subjects after Injection of rhGH.

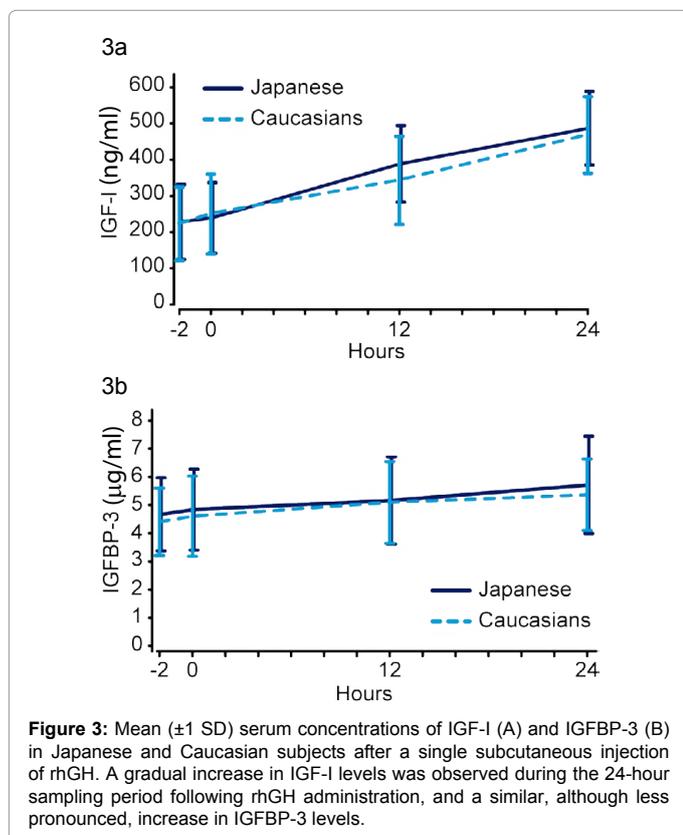


Figure 3: Mean (± 1 SD) serum concentrations of IGF-I (A) and IGFBP-3 (B) in Japanese and Caucasian subjects after a single subcutaneous injection of rhGH. A gradual increase in IGF-I levels was observed during the 24-hour sampling period following rhGH administration, and a similar, although less pronounced, increase in IGFBP-3 levels.

adverse events in the 24-hour somatostatin-infusion period after the rhGH bolus (Fischer’s exact test [two-sided], $p = 0.7545$). A total of 28 (76%) Japanese subjects reported 104 adverse events, and

31 (79%) Caucasian subjects reported 107 adverse events. Again, the majority of the adverse events reported were injection-site reactions (29% and 37% of the total number of adverse events in Japanese and Caucasian subjects, respectively) and somatostatin-related events (45% and 27%, respectively). There was no difference in the type and severity of the adverse events reported between Japanese and Caucasian subjects, or between the rhGH and placebo groups (the low number of placebo subjects should be considered).

No serious adverse events were reported following the administration of rhGH. One Japanese and one Caucasian subject were withdrawn because of nausea after somatostatin and rhGH administration; events that were assessed as unlikely related to the rhGH exposure. There were no differences between the rhGH and placebo groups in the types and severities of adverse events reported. No other clinically relevant changes in other safety parameters were reported.

Discussion

To our knowledge, this is the first study comparing the PK and PD of rhGH between Japanese and Caucasian subjects. The study demonstrated equivalence of the PK for rhGH (Norditropin®) for the two populations, and no apparent differences in levels of the basal circulating IGF-I and IGFBP-3 or the rhGH-mediated release of IGF-I and IGFBP-3 were observed between Japanese and Caucasian subjects. Furthermore, the somatostatin method was confirmed as being an efficient tool for suppressing the endogenous GH secretion. The safety profiles were all similar, reflecting the healthy subjects enrolled. Somatostatin-related adverse events were primarily gastrointestinal symptoms and hypoglycaemic events, which are well-known side-effects of somatostatin.

Equivalence of PK is based on an evaluation of the rate and extent of absorption of a drug substance. The individual importance of the three basic PK parameters AUC, C_{max} and t_{max} depends on the therapeutic indication for which the drug is intended, and the adverse-event profile of the drug [7]. For treatment with rhGH, the extent of absorption is of greater importance for PK and safety than the rate of absorption. In the present study, the concentrations of GH increased from the time of injection and reached maximum serum-concentrations around 4–5 hours in both Japanese and Caucasian subjects. This time frame is in keeping with previous studies in healthy subjects and hypophysectomised patients, showing serum peak-concentrations of GH after 3–6 hours [6-9].

Thus, PK equivalence for rhGH was indicated for Japanese and Caucasian adult subjects. The rhGH-mediated IGF-I and IGFBP-3 release was also similar between the two populations, a finding that corresponds well with the comparable beneficial effects of GH replacement on body composition observed for Japanese [10,11] and Caucasian [1-3,12,13] GH-deficient adults. The novel finding of similar IGF-I release in Japanese and Caucasian subjects after rhGH administration is also highly relevant in the context of using IGF-I as a GH-dependent biomarker to detect GH abuse [14,15].

In order to minimise the influence of endogenous GH, somatostatin was continuously administered by intravenous infusion, starting two hours prior to subcutaneous injection of the relatively high dose of rhGH (5 mg). Somatostatin suppresses the excretion of endogenous GH, and has been employed in rhGH bioequivalence studies in healthy volunteers [6,7]. The somatostatin method has an advantage over the involvement of hypophysectomised patients, as the disease complexity

in the latter population reduces the applicability of the observed PK to other settings. However, common side-effects of somatostatin administration are those affecting the gastrointestinal system such as abdominal discomfort and nausea, as previously described [7,16], but because of its short half-life, these effects are generally transient and disappear once the infusion is discontinued. Another more serious side-effect is hypoglycaemia as a result of the somatostatin-mediated inhibition of glucagon and insulin. In a study by Khan et al., a somatostatin infusion rate of 3 mg over 25 hours was used [6], and in another bioequivalence study investigating Norditropin®, the infusion rate was 120 µg/hour over 24 hours [7]. In the present study, four subjects experienced hypoglycaemic episodes within the first hour of somatostatin administration, and the infusion rate for all remaining subjects was therefore reduced from 120 to 50 µg/hour from 0 hours. The lower rate of 50 µg/hour was shown to suppress the endogenous GH secretion sufficiently. None of the four subjects experienced severe hypoglycaemia.

In conclusion, the results of this study have demonstrated equivalence of the PK for rhGH and similar levels of rhGH-mediated release of IGF-I and IGFBP-3 in healthy Japanese and Caucasian subjects, supporting the bridging of results obtained in clinical studies involving subjects of these origins.

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