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Exploration of the Effect and Pharmacokinetics of Intravenous and Subcutaneous GC1113, a Novel Erythropoiesis-Stimulating Agent

새로운 적혈구생성 촉진제 GC1113 의 정맥 또는 피하 투여시의 효과와 약동학적 특성에 대한 탐색 연구

2014년 2월

서울대학교 대학원

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한 혜 경

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이 논문을 의학석사 학위논문으로 제출함

2013년 10월

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한혜경의 의학석사 학위논문을 인준함

2013년 12월

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Abstract

Exploration of the effect and pharmacokinetics of intravenous and subcutaneous GC1113, a novel erythropoiesis-stimulating agent

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Introduction: GC1113, a hybrid Fc fused erythropoietin, is a novel erythropoiesis-stimulating agent which is expected to have an extended duration of action. The preclinical data showed that the hemoglobin increase lasted longer following GC1113 administration than it did following the administration of

NESP®(Darbepoetin alfa). This study aimed to investigate the pharmacodynamic (PD), pharmacokinetic (PK) characteristics and tolerability profiles of GC1113 in humans after single intravenous (IV) or subcutaneous (SC) administration and to compare the results with those for NESP®.

Methods: A dose-block randomized, placebo- and active- controlled, dose-escalation, phase 1 clinical trial was conducted with 96 healthy volunteers. Blood samples were collected before and up to 672 hours after drug administration and the erythropoietin concentration following the GC1113 or NESP® administration was measured by an enzyme-linked immunosorbent assay (ELISA). PK and PD parameters were determined using noncompartmental methods. Tolerability including immunogenicity evaluation was monitored during hospitalization and until the end of the study.

Results: The reticulocyte count-time profiles in the IV GC1113 3–5 μg/kg groups were comparable with those of the NESP® 30 μg. After subcutaneous administration of GC1113, reticulocyte count peaked later and decreased more slowly than it did following NESP® administration. For pharmacokinetics, GC1113 showed faster elimination and slower absorption through subcutaneous administration than NESP®. The GC1113 (0.3–5 μg/kg for IV, 1–8 μg/kg SC) was well tolerated in the volunteers, and no immunogenicity was observed.

Conclusions: GC1113 showed erythropoietic activity in healthy volunteers. Intravenous GC1113 showed comparable erythropoietic activity to NESP®, and

following subcutaneous administration, the reticulocyte count increase lasted longer for GC1113 than for NESP®. GC1113 was tolerated and effective in the studied dose range; these findings could be applied to further clinical studies with patients.

Keywords: Pharmacokinetics, pharmacodynamics, erythropoiesis, healthy volunteers

Student number: 2012-21776

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AE	Adverse event
AUC	Area under the concentration-time curve
AUC_{inf}	AUC from the drug administration to time infinity
AUC_{last}	AUC from the drug administration to the last measurable concentration
C_{max}	Maximum observed plasma concentration
$\Delta AUEC_{last}$	The area under the baseline corrected pharmacodynamic value-time curve from time 0 to the last measurable value
ΔE_{max}	The maximum baseline corrected pharmacodynamic value
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
EPO	Erythropoietin
IgG	Immunoglobulin G
IV	Intravenous
LLOQ	Lower limit of quantification
PD	Pharmacodynamics
PK	Pharmacokinetics

rHuEPO	Recombinant human erythropoietin
SC	Subcutaneous
SD	Standard deviation
sTfR	soluble transferrin receptor
T_{max}	Observed time of maximum plasma concentration

INTRODUCTION

Erythropoietin (EPO) is a glycoprotein that is mainly produced in the kidneys in response to hypoxia, and it plays a crucial role in producing and maturing erythrocytes [1]. EPO stimulates the maturation of erythrocytes by binding to the EPO receptor on the surfaces of erythroid precursor cells in bone marrow [2]. After gene cloning was succeeded in 1985 [3, 4], recombinant human EPOs (rHuEPOs) were produced and used in clinical settings [5]. rHuEPOs have been used to treat the anemia with chronic kidney disease and cancer chemotherapy [6]. Since the first recombinant EPO (Epoetin alfa) was developed, a variety of long-acting and modified EPO derivatives have been introduced to the market [7]. Darbepoetin alfa (NESP®) is a hyperglycosylated rHuEPO that contains 2 additional carbohydrate chains, resulting in a longer elimination half-life [8]. Mircera®, methoxy polyethylene glycol-epoetin beta, has a prolonged half-life because of PEGylation [9]. In dialysis patients, intravenous (IV) NESP® has a half-life of 25 hours, which is 3 times longer than that of the first-generation rHuEPOs [10]. The half-life of Mircera® was approximately 130 hours with IV or subcutaneous (SC) administration [11].

GC1113 is a novel erythropoiesis-stimulating agent that is under development. GC1113 combines natural EPO with a hybrid Fc using continuous protein antibody fusion technology. Its molecular weight is approximately 113 kDa, which is 3 times

larger than that of NESP® at equipotent molecule. It is known that fusion with Fc portion of immunoglobulin G (IgG) can increase the serum half-life of therapeutic monoclonal antibodies [12]. Based on this finding, GC1113 was developed to increase the serum half-life through fusion of EPO with hybrid Fc portion, consisting of IgG4 and IgD. Through its increased half-life, GC1113 should be more convenient to administer via the IV or SC route for anemic patients.

The preclinical data showed that GC1113 had comparable or longer elimination half-lives, and its effect on erythropoiesis lasted longer than that of NESP®. In normal and acute renal failure-induced rats, both IV and SC GC1113 showed erythrocyte stimulation. The maximum hemoglobin levels of normal rats after IV administration were 16.7 g/dL for GC1113 and 16.3 g/dL for equipotent NESP®. The maximum hemoglobin levels after SC administration were 16.6 g/dL for GC1113 and 15.2 g/dL for equipotent NESP®. In monkeys, GC1113 showed a longer half-life than NESP® for both IV and SC administrations (approximately 1.6-fold for IV and 3-fold for SC). It was well tolerated during toxicology testing in rats and monkeys for single and multiple administrations (unpublished data).

The objectives of this study were to evaluate the pharmacodynamic (PD), pharmacokinetic (PK) and tolerability of GC1113 for both IV and SC administrations in healthy male volunteers. These assessments could help to decide the optimal dosage regimen for later phase clinical trials with anemic patients. In addition, the PD and PK characteristics of GC1113 were compared to those of an

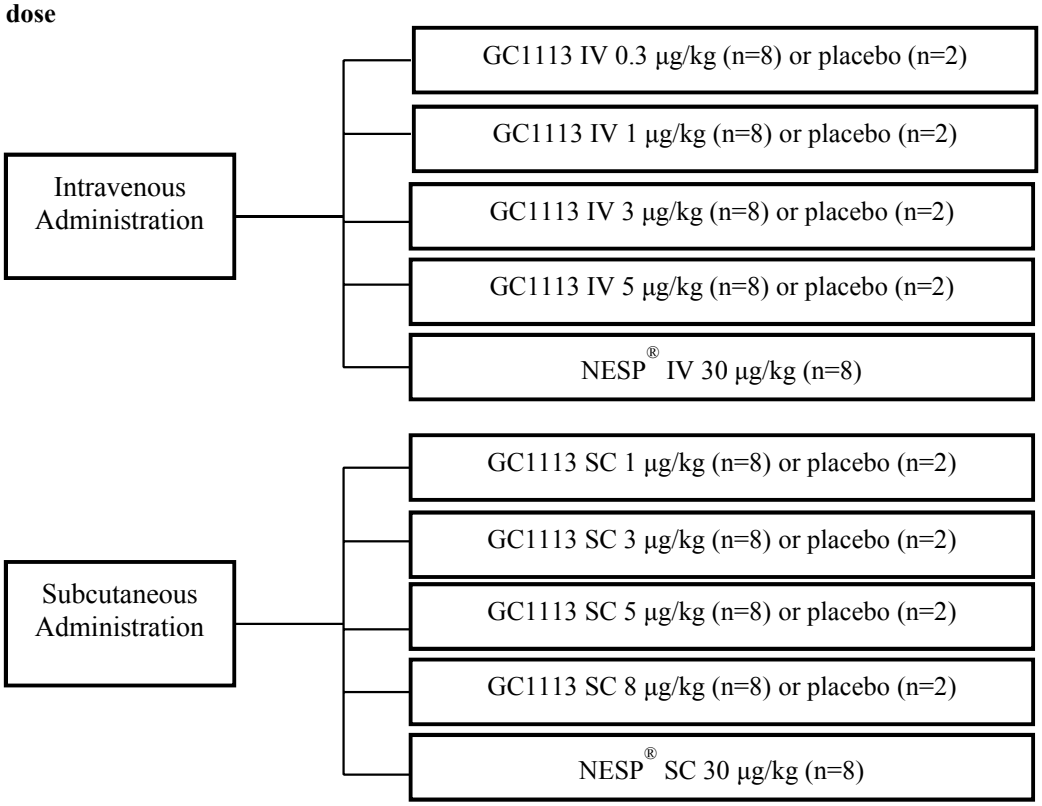
active control, NESP®, for both administration routes.

MATERIALS AND METHODS

Study design

This study was conducted in 2 parts; IV administration and SC administration. In each part, a dose block-randomized, double-blind, single-dose, dose-escalation study was performed for GC1113 and a placebo. An Intravenous 0.3, 1, 3, or 5 μg/kg dose of GC1113 or a 1, 3, 5, or 8 μg/kg dose of SC GC1113 was administered. For the active control in each part, a single dose of 30 μg of NESP® was administered in an open-label manner. The subject disposition data are summarized in Figure 1. The study was conducted at the Clinical Trials Center of Seoul National University Hospital (SNUH) between June 2011 and July 2012. The study protocol was approved by the SNUH Institutional Review Board. All study procedures were performed in accordance with the Declaration of Helsinki and the guidelines for Good Clinical Practice (ClinicalTrials.gov Identifier: NCT01363934).

Figure 1. Subject disposition by route of administration, investigational drugs and its dose



All study participants provided written informed consent before the eligibility screening test was conducted. After the study on each dose group of GC1113 was completed, the interim safety analysis of the prior dose group was performed by an independent data monitoring committee. Iron is needed in erythropoiesis, and iron deficiency can limit erythropoietic activity. To evaluate erythropoietic activity (independent of iron levels), oral iron supplements were provided to subjects with ferritin levels under 100 μg/L during the study period. To evaluate the PK and PD, blood samples were collected before administering the drug and up to day 29 after the drug administration (at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96, 120, 168, 240, 336, 504, and 672 hours for the PK and at 4, 8, 12, 24, 48, 60, 72, 96, 120, 168, 240, 336, 504, and 672 hours for the PD).

Pharmacodynamic assessments

The reticulocyte count, hemoglobin, and reticulocyte-hemoglobin content for each subject were collected for the PD assessments. The reticulocyte-hemoglobin content was obtained by measuring the amount of hemoglobin contained in the reticulocytes to provide an indirect measurement of the functional iron available for erythrocyte formation [13]. In addition, iron store status of each subject was determined to evaluate erythropoiesis by measuring levels of ferritin, soluble transferrin receptor (sTfR), and transferrin saturation.

To more accurately estimate the inter-individual variability, the measured PD values were corrected by the individual baseline PD values, and then a noncompartmental analysis was performed. The maximum baseline corrected PD value (ΔE_{max}) was determined from the observed data. The area under the baseline corrected PD value-time curve from time 0 to the last measurable value ($\Delta AUEC_{last}$) was calculated using the linear trapezoidal summations. To evaluate the time course of the reticulocyte count, the $\Delta AUEC_{last}$ from the time of the drug administration to 2 weeks and 4 weeks after the drug administration were compared.

Determining the erythropoietin concentration

To determine the erythropoietin concentration following the GC1113 or NESP® administration, an enzyme-linked immunosorbent assay (ELISA) using the Human EPO Immunoassay kit (R&D Systems Inc., Minneapolis, MN) was used. The same analytic method was used for all groups throughout the study, but the lower limits of quantification (LLOQs) were different among the groups. The LLOQ of IV administration group was 1.56 ng/mL. To precisely determine the PK profiles of SC, LLOQs were established lower in SC than those of IV. The LLOQs were 156.25 pg/mL for SC GC1113 1, 3, and 5 μg/kg dose groups and 78.125 pg/mL for SC GC1113 8 μg/kg dose group and NESP® 30 μg group. The coefficient of correlation (γ^2) was 0.998 or

greater for this set of analyses.

Pharmacokinetic assessments

The individual serum concentration-time profiles for each subject and the mean concentration values according to the sampling time were plotted. Non-compartmental method was employed to analyze the individual PK parameters using Phoenix® (version 1.2; Pharsight Corporation, Sunnyvale, CA). The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were determined directly from the observed values. The terminal elimination rate constant (λ_z) was calculated by linear regression of the terminal slope of the log-transformed individual plasma concentration-time data. The effective half-life was calculated as ln(2)•MRT, which MRT (mean residence time) is the average amount of time that the drug resides in the body. We calculated the effective half-life because detecting endogenous EPO influence the estimation of λ_z. The area under the concentration-time curve (AUC) from time 0 to the last measurable concentration (AUC_{last}) was calculated using the linear trapezoidal and log- linear trapezoidal summations for the increasing and decreasing phases of the individual plasma concentration-time curve, respectively. The AUC from time 0 to infinity (AUC_{inf}) was calculated using the following formula: $AUC_{inf} = AUC_{last} + C_t/ \lambda_z$, where C_t is the last plasma concentration measured. To evaluate the dose-linearity, the comparisons of

dose-normalized C_{max} and AUC_{last} were performed using Kruskal-Wallis test. Because of its lower LLOQ, endogenous EPO was detected in SC administration group. For the SC group, baseline corrected and dose-normalized PK parameters were used to evaluate the dose-linearity. To evaluate the PK-PD relationship, the PK parameters (C_{max}, AUC_{last}) and PD parameters of the reticulocyte counts (ΔE_{max}, $\Delta AUEC_{last}$) were compared using Pearson's correlation analysis.

Tolerability

Safety profiles were monitored during hospitalization and until the end of the study using laboratory tests (hematology, chemistry, coagulation and urinalysis), vital signs, 12-lead electrocardiograms, spleen sonography, and knee radiography, and the determination of adverse events (AEs). AEs were monitored by asking subjects general health-related questions at the scheduled physical examinations and by self-reporting from the subjects during the study. In addition, for the GC1113 administration group, an immunogenicity evaluation was conducted using ELISA with the GC1113 Ab ELISA Q Kit (BioNote Inc., Korea). Samples for the GC1113 antibody test were collected prior to and at 15 and 29 days after the GC1113 administration.

Results

Pharmacodynamic analysis

A total of 100 healthy Korean subjects received GC1113 (or placebo) or NESP®, and 96 subjects completed the study (1 subject, withdrawal of consent; 1 subject, AE – ear abscess; 2 subjects, lost to follow-up). The baseline corrected reticulocyte count was evaluated as a primary PD parameter. After drug administration with either GC1113 or NESP®, the baseline corrected reticulocyte count increased compared with the placebo group (Figure 2). When the same dose was administered via different administration routes, the $\Delta AUEC_{last}$ of the SC groups increased more than the IV group for both GC1113 and NESP®. GC1113 and NESP® showed similar timing of their reticulocyte count change patterns after the IV administration. The IV GC1113 3–5 μg/kg groups showed similar PD-time profiles compared to the NESP® 30 μg group. After SC administration, the PD time courses of GC1113, including the time to reach the maximum reticulocyte count, were different among the SC dose groups. After 2 weeks from drug administration, the change in the reticulocyte count increased as the dose increased in IV administration. In SC administration, little difference was observed among the dose groups, and great variability was observed 4 weeks after drug the administration (Figure 3).

There was no dose-related trend in the hemoglobin, reticulocyte-hemoglobin content, and transferrin saturation values (Figure 4). Ferritin appeared to decrease and return to baseline, and sTfR appeared to increase and then return to baseline, both dependent on the dose administered (Figure 5, 6).

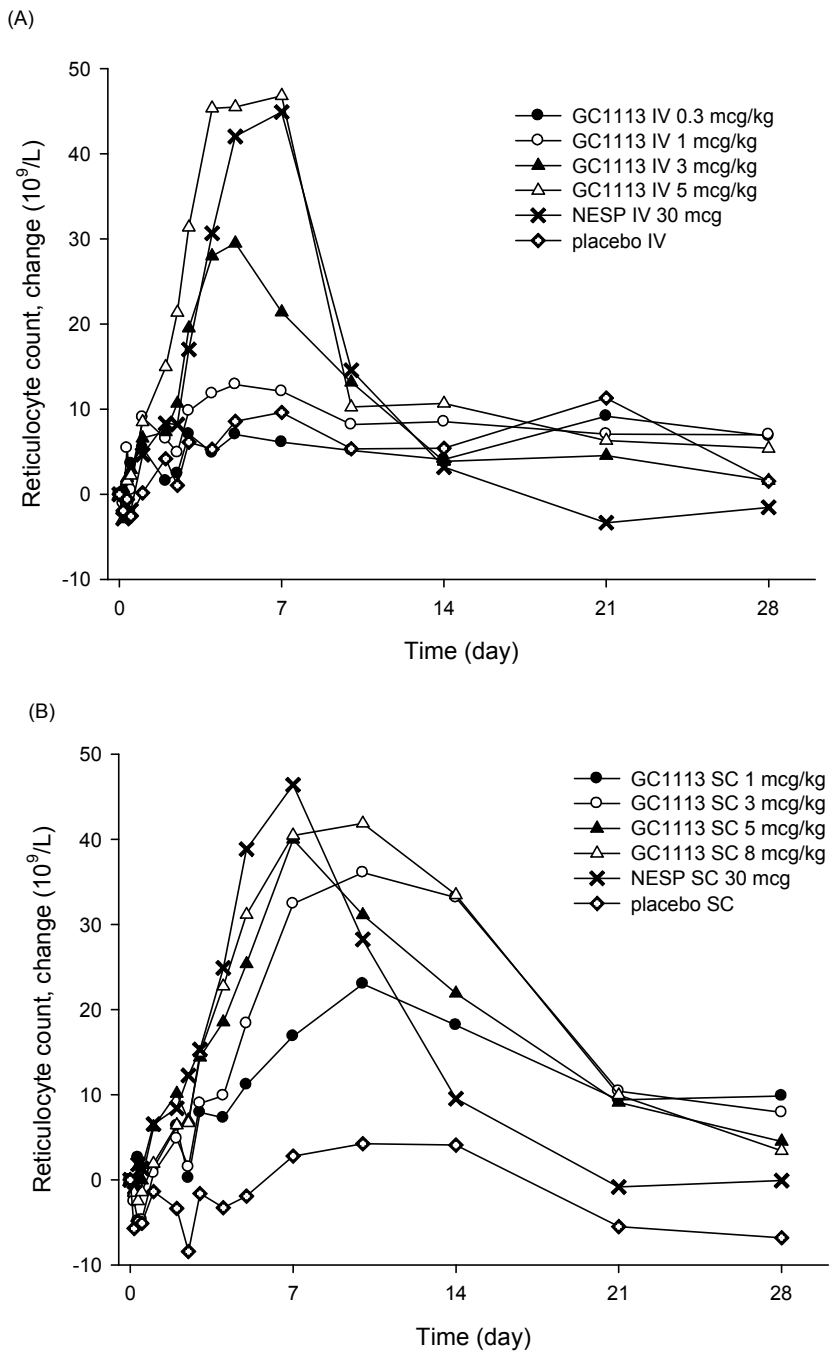


Figure 2. Mean reticulocyte count change from baseline–time profiles up to 672 hours (Day 29) after a single administration of GC1113, NESP® or placebo. (A) intravenous administration, (B) subcutaneous administration

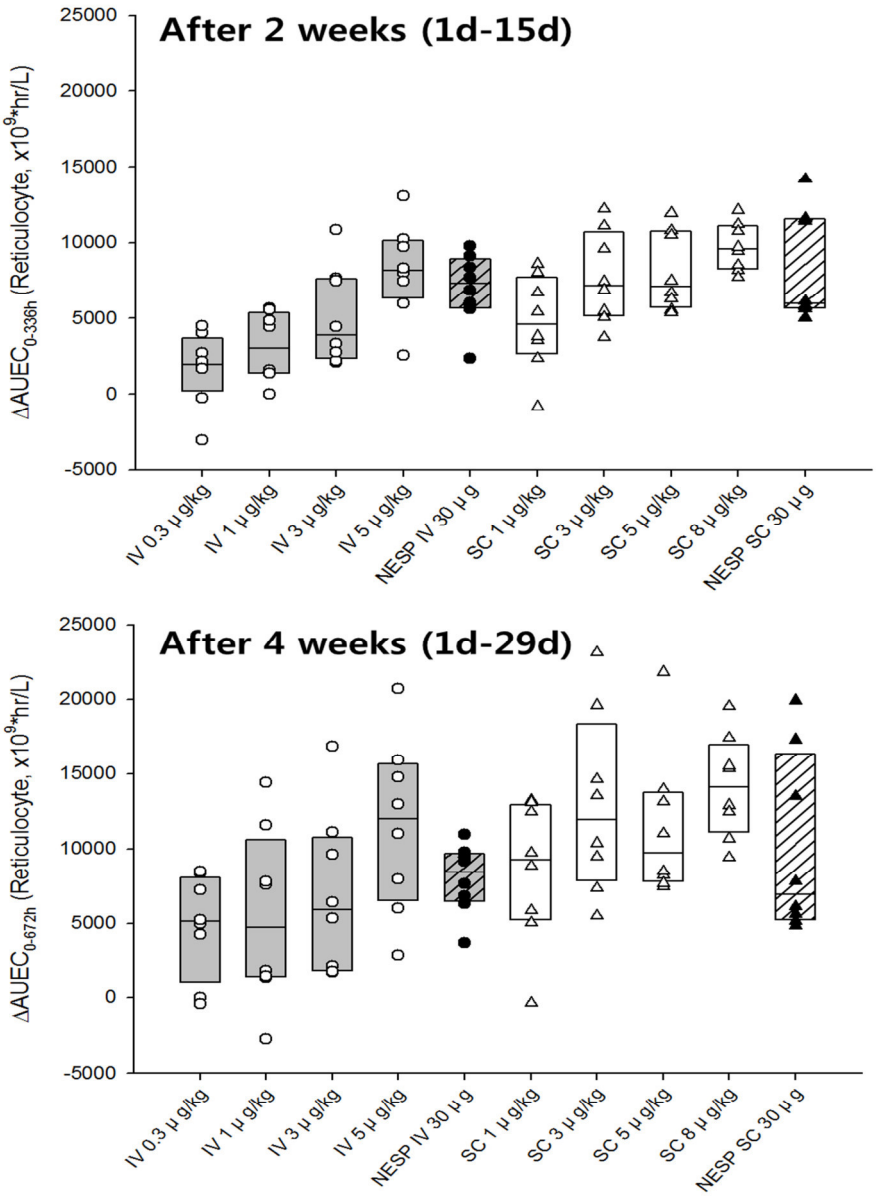


Figure 3. Individual baseline corrected AUECs (area under the reticulocyte count value -time curve) up to 2 weeks and 4 weeks after a single administration of GC1113 or NESP® (GC1113 IV 0.3, 1, 3, 5 μg/kg, NESP® IV 30 μg, GC1113 SC 1, 3, 5, 8 μg/kg, NESP® SC 30 μg)

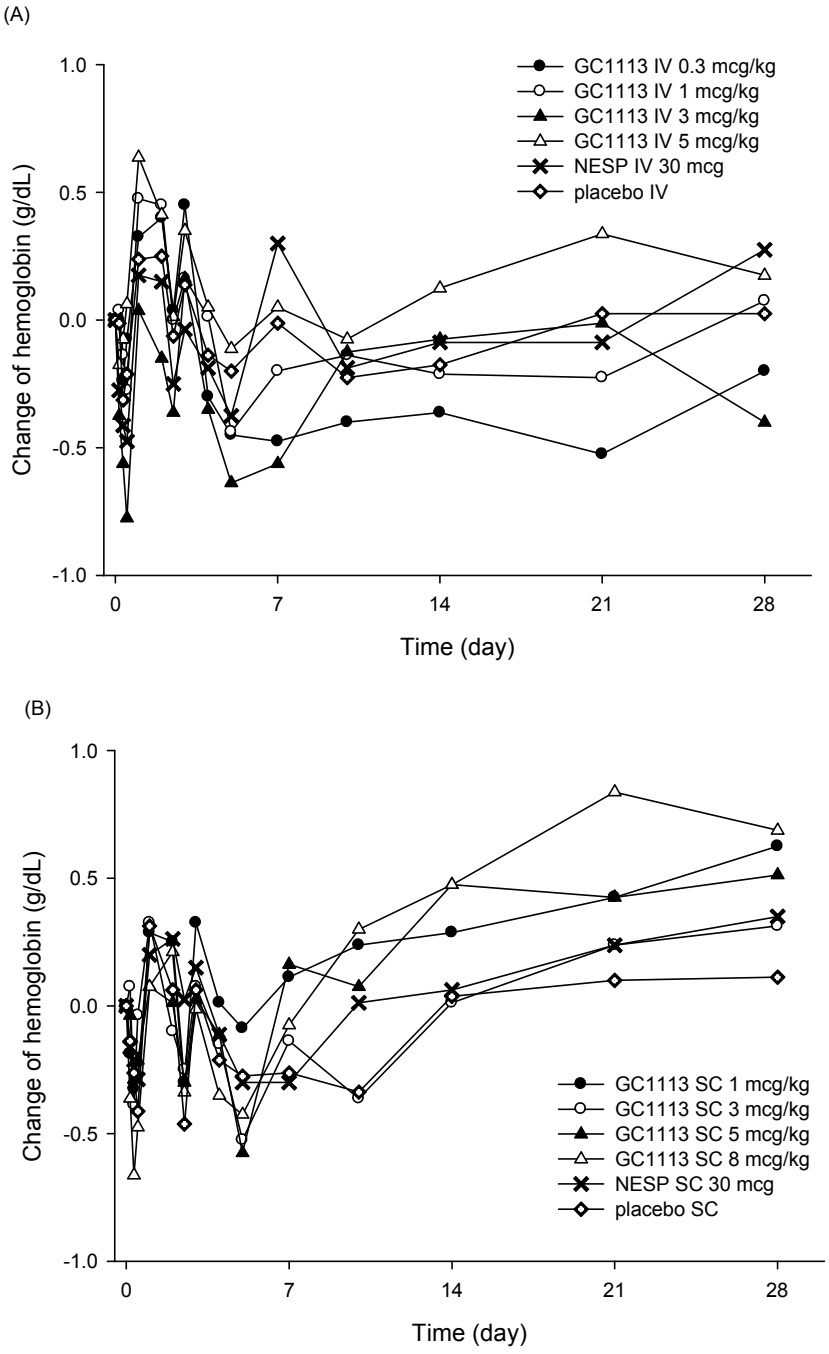


Figure 4. Mean hemoglobin change from baseline–time profiles up to 672 hours (Day 29) after a single administration of GC1113 or NESP® or placebo (A: intravenous administration, B: subcutaneous administration)

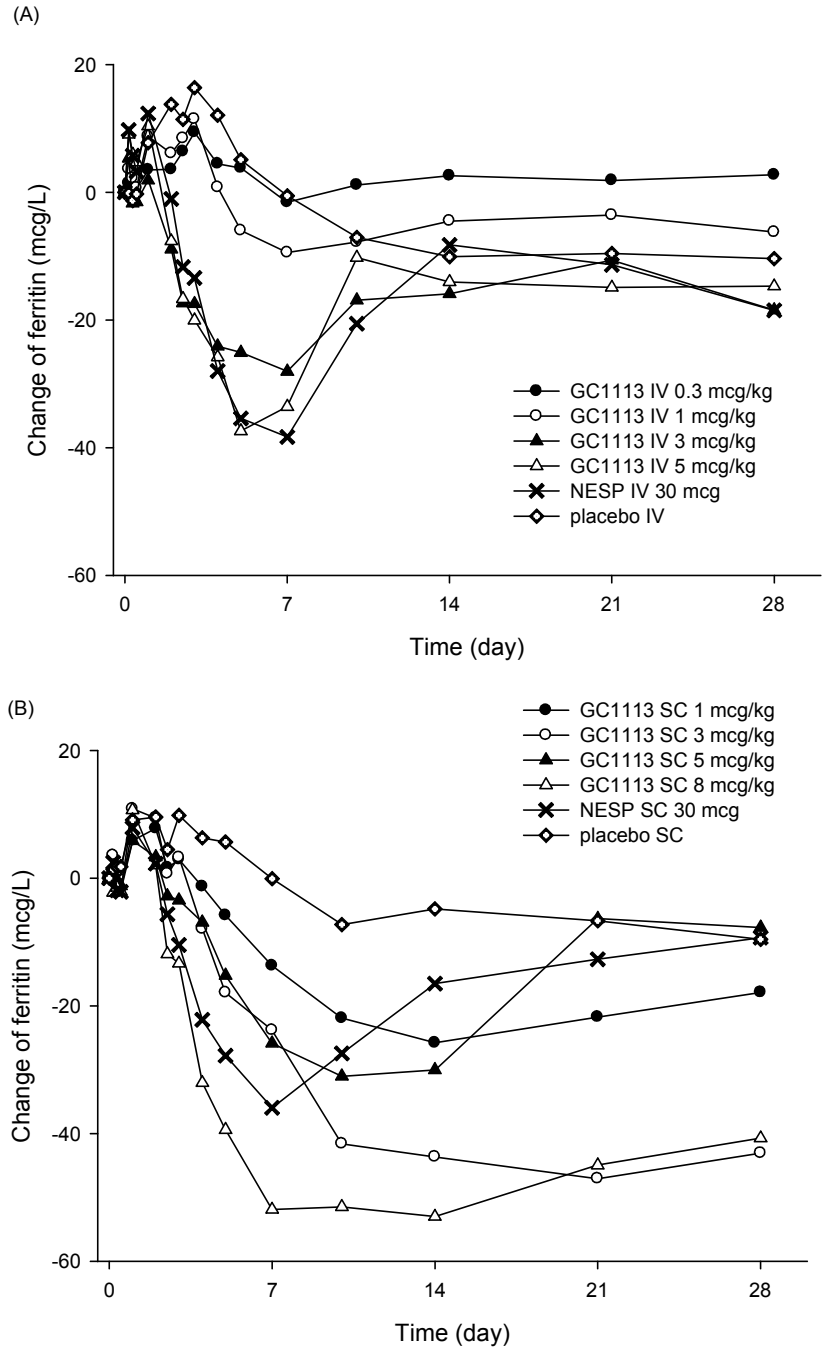


Figure 5. Mean ferritin change from baseline–time profiles up to 672 hours (Day 29) after a single administration of GC1113 or NESP® or placebo (A: intravenous administration, B: subcutaneous administration)

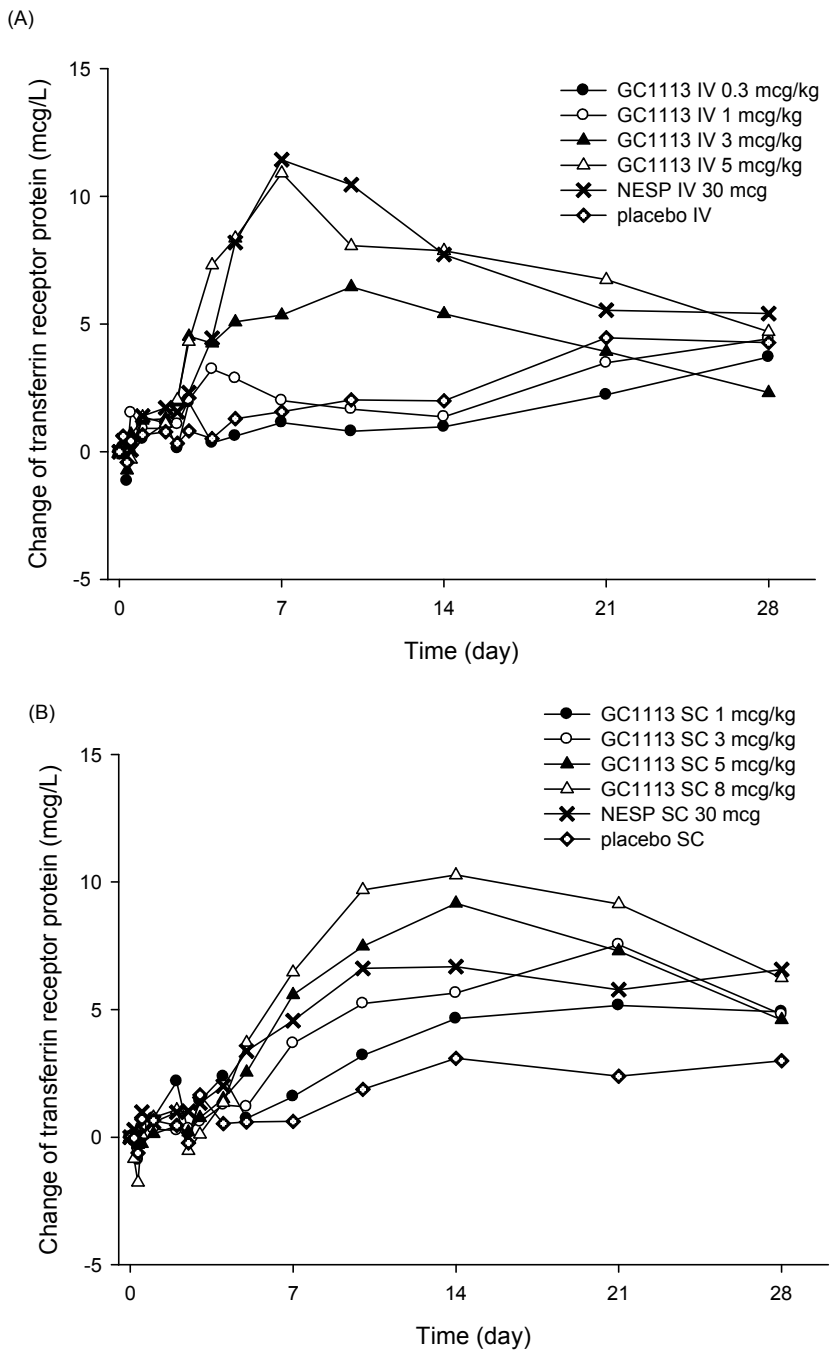


Figure 6. Mean transferrin receptor protein change from baseline –time profiles up to 672 hours (Day 29) after a single administration of GC1113 or NESP® or placebo (A: intravenous administration, B: subcutaneous administration)

Pharmacokinetic analysis

The C_{max} and AUC_{last} of IV NESP® 30 μg were closest to those of IV GC1113 at 1 μg/kg, but the effective half-life was longer for IV NESP® 30 μg than for IV GC1113 at 1 μg/kg. The effective half-life of NESP® 30 μg was similar to that of IV GC1113 3–5 μg/kg (Figure 7, Table 1, 2). All SC GC1113 (1–8 μg/kg) groups and SC NESP® 30 μg group showed similar effective half-lives. Based on the Kruskal-Wallis test results, the dose-normalized C_{max} indicated that C_{max} increased proportionally with increasing dose ($P = 0.264$); however, the dose-normalized AUC_{last} values were different among the dose groups for IV administration ($P < 0.001$) (Figure 8). For the SC group, baseline corrected and dose-normalized C_{max} and AUC_{last} did not differ from the dose groups ($P = 0.730$ for C_{max}, $P = 0.102$ for AUC_{last}) (Figure 9).

Table 1. Summary of pharmacokinetic and pharmacodynamic parameters following intravenous administration of GC1113 or NESP®

	Intravenous administration				
Drug	GC1113				NESP®
Dose	0.3 μg/kg (n=8)	1 μg/kg (n=8)	3 μg/kg (n=8)	5 μg/kg (n=8)	30 μg (n=8)
Pharmacokinetic parameters					
C_{max} (μg/L)	4.82 (1.4)	18.99 (3.47)	51.41 (16.8)	88.09 (15.33)	12.17 (1.71)
AUC_{last} (hr•μg/L)	21.32 (12.07)	147.61 (57.76)	826.32 (342.93)	1759.72 (315.09)	210.16 (35.36)
AUC_{inf} (hr•μg/L)	39.82 (10.78)	191.33 (59.51)	877.60 (323.03)	1799.65 (313.28)	264.75 (38.78)
Effective half-life (hr)	1.99 (0.89)	3.87 (1.02)	7.98 (1.92)	10.51 (1.34)	9.29 (0.86)
Pharmacodynamic parameter					
$\Delta AUEC_{last}$ (10^9•hr/L)	4781.60 (3452.15)	5423.35 (5853.46)	6879.04 (5370.52)	11540.14 (5769.97)	7986.48 (2340.06)

Data are presented as mean (standard deviation)

C_{max} (maximum concentration), AUC (Area under the concentration-time curve), AUC_{last} (AUC from time 0 to the last measurable concentration), AUC_{inf} (The AUC from time 0 to infinity), $AUEC_{last}$ (Area under the baseline-corrected reticulocyte count -time curve)

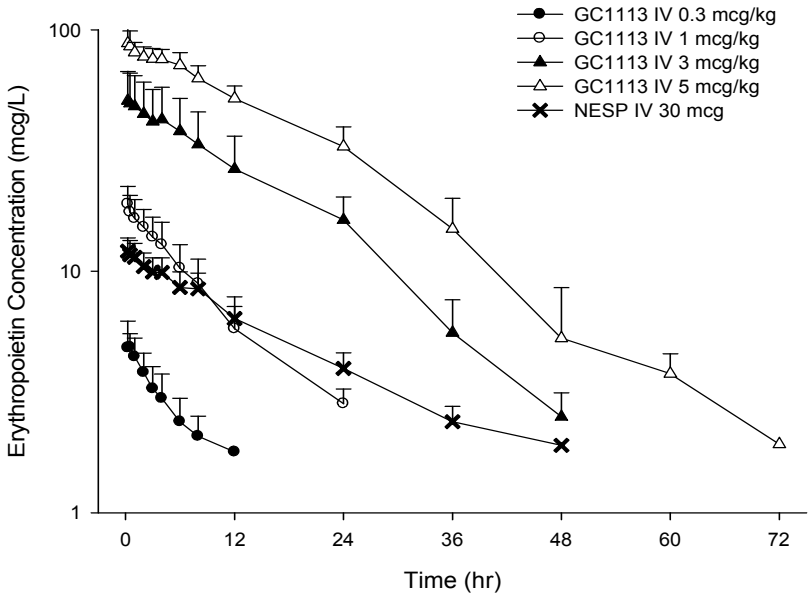
Table 2. Summary of pharmacokinetic and pharmacodynamic parameters following subcutaneous administration of GC1113 or NESP®

	Subcutaneous administration				
Drug	GC1113				NESP®
Dose	1 μg/kg (n=8)	3 μg/kg (n=8)	5 μg/kg (n=8)	8 μg/kg (n=8)	30 μg (n=8)
Pharmacokinetic parameters					
C_{max} (μg/L)	0.87 (0.45)	1.72 (1.26)	2.81 (1.88)	5.71 (4.8)	1.07 (0.34)
AUC_{last} (hr•μg/L)	198.46 (106.27)	287.46 (102.65)	443.24 (73.23)	615.97 (147.05)	216.48 (52.47)
AUC_{inf} (hr•μg/L)	341.74 (179.32)	374.97 (83.07)	514.52 (54.12)	716.07 (136.88)	315.00 (101.58)
Effective half-life (hr)	157.88 (33.13)	151.58 (35.79)	145.89 (37.3)	138.45 (36.43)	157.33 (13.09)
Pharmacodynamic parameter					
$\Delta AUEC_{last}$ (10^9•hr/L)	8480.03 (4740.09)	12935.85 (6037.02)	11480.05 (4863.71)	14140.63 (3412.19)	9983.65 (5940.2)

Data are presented as mean (standard deviation)

C_{max} (maximum concentration), AUC (Area under the concentration-time curve), AUC_{last} (AUC from time 0 to the last measurable concentration), AUC_{inf} (The AUC from time 0 to infinity), $AUEC_{last}$ (Area under the baseline-corrected reticulocyte count -time curve)

(A)


GC1113 IV 0.3 mcg/kg
GC1113 IV 1 mcg/kg
GC1113 IV 3 mcg/kg
GC1113 IV 5 mcg/kg
NESP IV 30 mcg
Erythropoietin Concentration (mcg/L)
100
10
1
0
12
24
36
48
60
72
Time (hr)

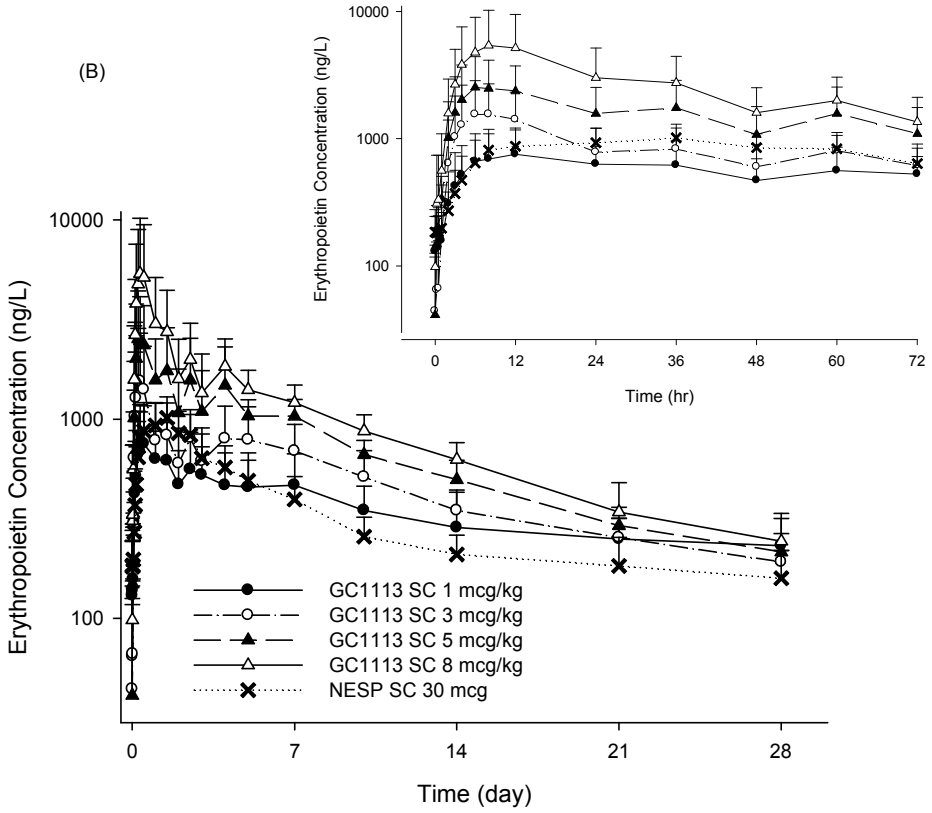


Figure 7. Mean serum concentration-time profiles of GC1113 or NESP® after single (A) intravenous and (B) subcutaneous administration of 0.3, 1, 3, 5 µg/kg of GC1113 or 30 µg of NESP®. Inset shows the initial 72 hours portion of the profile. (log-linear scale)

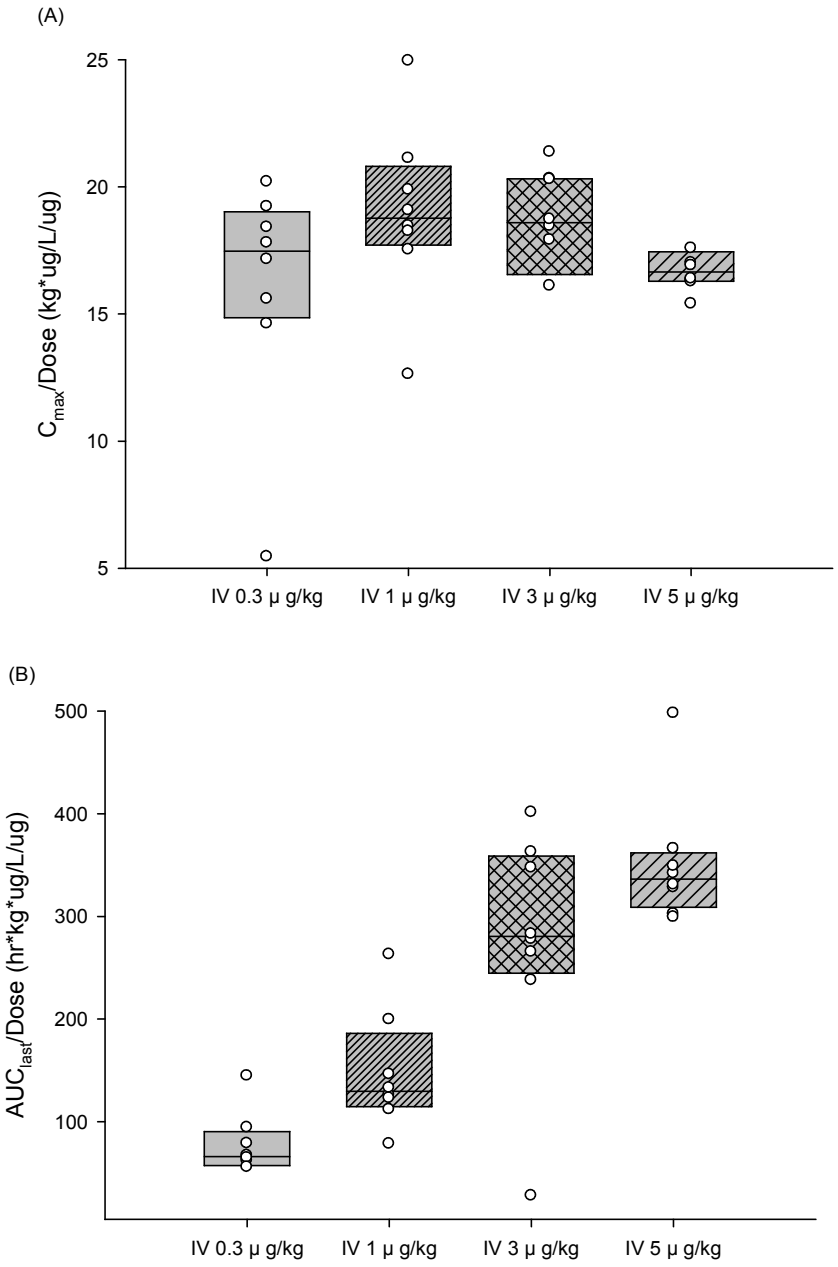


Figure 8. Individual Dose-normalized C_{max} and AUC_{last} values by intravenous GC1113 treatment. Open circle represents the individual values. Box plot provides median and 25%/75% quartiles.

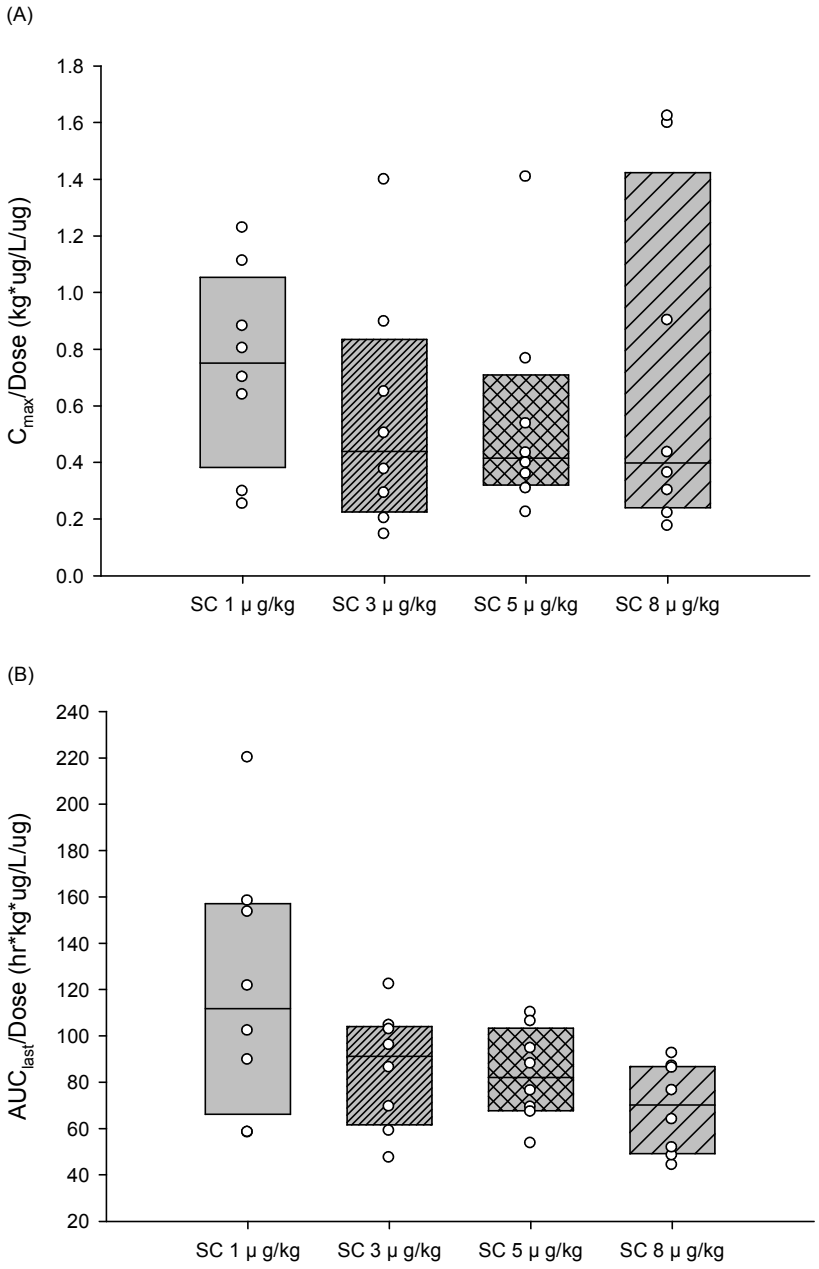


Figure 9. Individual Dose-normalized, baseline corrected C_{max} and AUC_{last} values by subcutaneous GC1113 treatment. Open circle represents the individual values. Box plot provides median and 25%/75% quartiles.

Investigating the relationship between the PK parameters (C_{max}, AUC_{last}) and PD parameters (reticulocyte count ΔE_{max}, $\Delta AUEC_{last}$) using Pearson's correlation analysis, the IV groups showed a positive and statistically significant (*P*-value < 0.05) correlation between the PK and PD parameters. However, the SC groups did not demonstrate linearity between their PK and PD parameters (Figure 10).

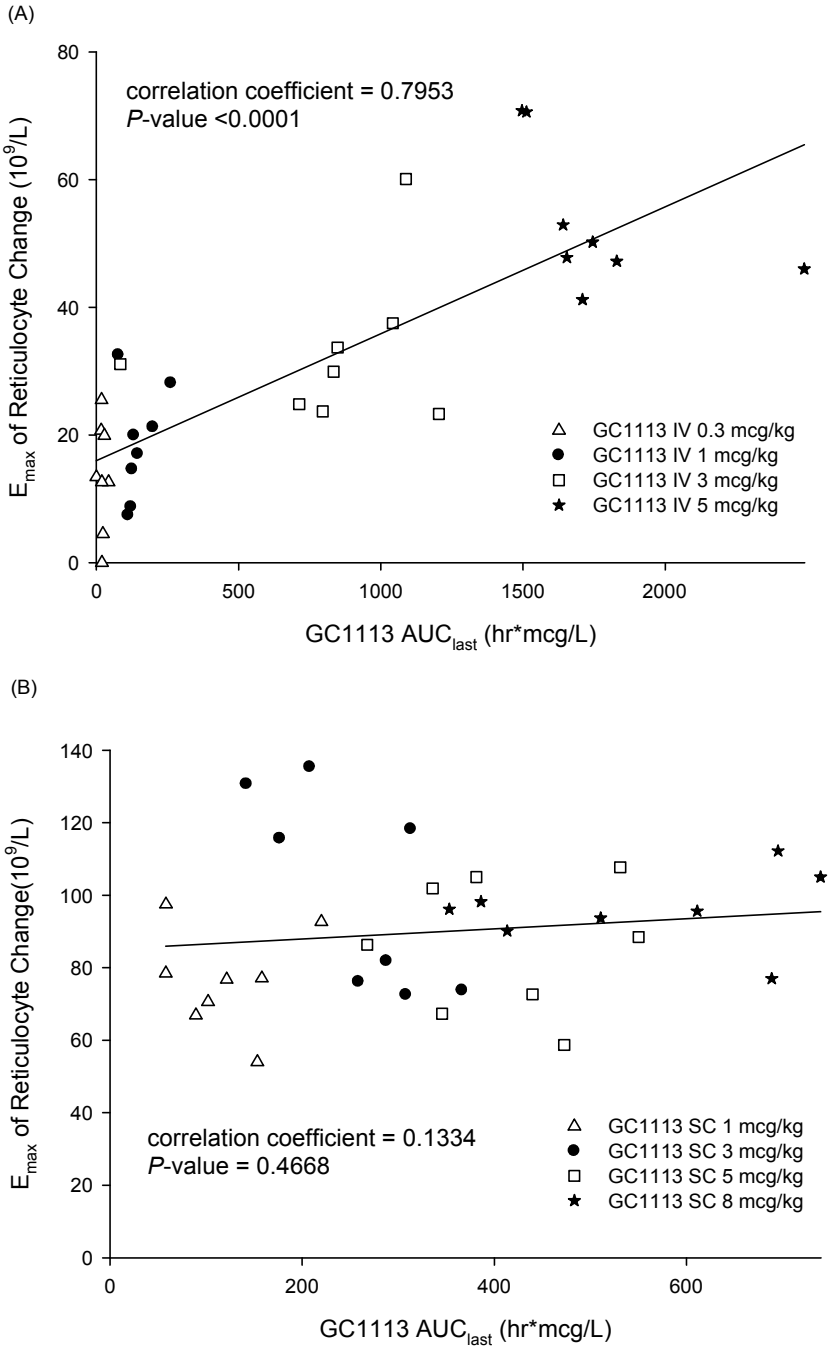


Figure 10. Pharmacokinetic-pharmacodynamic relationship of AUC_{last} (area under the time-concentration curve from time 0 to last measurable concentration) vs E_{max} (maximum observed reticulocyte) of baseline corrected reticulocyte after

single GC1113 administration. (A) intravenous administration, (B) subcutaneous administration

Tolerability

Among the 100 subjects, 39 experienced 70 adverse events. Among the adverse events that were related to GC1113, headache was the most frequently reported (6 cases), followed by 3 cases each of rhinorrhea and upper respiratory tract infection. All adverse events were mild or moderate in intensity, and the subjects recovered. There was no serious adverse event. In addition, there were no clinically significant changes in the laboratory tests, vital signs, ECGs, spleen sonography, or knee radiography. Iron was provided as needed to the 96 subjects with ferritin levels under 100 μg/L during the clinical trial. No GC1113 antibody formation was detected throughout the study period.

Discussion

This study explored the effectiveness and tolerability of GC1113 in healthy volunteers. GC1113 showed erythropoietic activity based on not only in the reticulocyte count increment but also on other PD and iron parameters, such as ferritin and sTfR. Because this study was the first to observe GC1113 administration in humans, the safety profiles were intensively monitored during the study. No immunogenicity was found after a single administration in this study as reported for other Fc fusion drugs [14].

The reticulocyte count increased after the IV and SC administration of GC1113. The IV formulation of 3–5 μg/kg GC1113 showed PD profiles similar to those of 30 μg of NESP®. After the SC administration of GC1113, the reticulocyte count level peaked later and decreased more slowly compared with the administration of NESP®. The SC GC1113 is expected to have longer dosing interval than NESP®. Considering reticulocyte count-time profile (Figure 2), reticulocyte counts returned to its baseline value after 2 weeks in IV administration and it took 4 weeks to return baseline value in SC administration. For IV administration, the dose-response relationship was observed much obviously after 2 weeks than 4 weeks from drug administration (Figure 3). For SC administration, the dose-response relationship can be determined after 4 weeks from drug administration. After

4 weeks, the $\Delta AUEC_{last}$ increased sub-proportionally to the dose increase and the great inter-individual variability is observed both SC GC1113 and NESP®. Further evaluations in anemic patients are needed to confirm the dose-response of GC1113.

When considering the PD parameters all together including reticulocytes, GC1113 increased the erythropoietic activity in humans and is expected to be effective in anemic patients. In addition to the reticulocyte count change, the ferritin level was decreased, and the sTfR was increased after the GC1113 and NESP® administration, and then it returned to baseline levels. Ferritin reflects the status of iron, which is required to form erythrocytes [15]. Increased sTfR levels indicate increased erythropoietic activity or tissue iron deficiency [16]. During the study, the subjects' iron levels were monitored and supplemented to prevent deficiency. Thus, the sTfR increase in this study represented erythropoietic activity.

In terms of PK, the effective half-life of GC1113 was slightly shorter than NESP® when delivered via IV and longer than NESP® when delivered SC. The terminal slope from the concentration-time profile of GC1113 was steeper for IV administration and flatter for SC administration compared with NESP®. After SC administration, the drug absorption continued even in the elimination phase, and the SC elimination phase was presumably affected by not only the elimination but also the slow absorption rate [17]. This can explain why the effective half-life was longer after SC administration, despite

GC1113's elimination by a common pathway regardless of the administration route. In addition, SC GC1113 showed slower absorption than SC NESP®. The molecular size of GC1113 is 3 times larger than NESP®, which could be the reason for the slower absorption following the SC administration of GC1113 compared with NESP®. There may also be some relationship between the existence of the Fc portion and the SC absorption rate; however the effect of the Fc portion on SC bioavailability is not yet known [18].

The elimination mechanism of EPO has not been fully elucidated to date, but it is known that EPO receptor mediated endocytosis degradation is a main elimination pathway [19, 20]. Because of the saturation of the receptor-mediated endocytosis elimination pathway, the PK of GC1113, which is an EPO derivative, was expected to be nonlinear. IV GC1113 was judged to have nonlinear PK characteristics based on the Kruskal-Wallis test results using the dose-normalized AUC_{last} and the increase in the effective half-life with the increased dose. For IV administration, the AUC_{last} increased supra-proportionally to the dose increase. However, the dose-normalized C_{max} and AUC_{last} of SC GC1113 were not different among the 1–8 μg/kg dose groups. Because the SC elimination phase was affected by slow absorption, C_{max} and AUC_{last} were less affected by saturation of the receptor-mediated endocytosis pathway. Considering the PK and PD parameters together, a positive correlation was observed, particularly in the relationship between the AUC_{last} and ΔE_{max} in the IV administration group. There was no obvious correlation

between the PK and PD in the SC administration group (Figure 10). The time delay between the EPO concentration and the response in the erythrocyte counts is known[21], and this relationship was also observed in this study. We evaluated only the linear relationship of the PK and PD parameters. Further evaluation using a time delay model to consider an indirect PK-PD relationship would be helpful to understand the relationship between PK and PD.

The terminal half-life of NESP® in this study was shorter than that in other published studies; in previous reports on renal disease or cancer patients, the terminal half-life of NESP® was approximately 25-33 hours following IV administration and approximately 49–105 hours following SC administration [10, 22-24]. The terminal half-life of NESP® in this study (mean ± standard deviation) was 13.0 ± 4.7 hours for IV administration and 79.7 ± 38.3 hours for SC administration. Chemotherapy can reduce the clearance of NESP®[25], because bone marrow contributes to the mechanism of cell-mediated clearance of erythropoietin [26]. Thus, patients undergoing chemotherapy might show longer half-lives than healthy volunteers.

There were some limitations to making direct comparisons of the PK characteristics of GC1113 and NESP®. It is not known whether the Fc portion of GC1113 separates after administration. It was also difficult to separately quantify the endogenous and exogenous EPO levels within the total measured EPO. These factors impeded direct comparisons of the PK characteristics

between GC1113 and NESP®. However, the increased reticulocyte count could be compared between GC1113 and NESP®, and GC1113 showed comparable efficacy to NESP®.

This study evaluated the PD, PK and tolerability of a novel erythropoiesis-stimulating agent using IV and SC formulations. IV GC1113 showed comparable erythropoietic activity to NESP®, and following SC administration, the reticulocyte count increase lasted longer for GC1113 than for NESP®. GC1113 was well tolerated, and it was effective in the studied dose range. These results could be applied to further clinical studies in anemia patients with chronic kidney disease and in cancer patients undergoing chemotherapy.

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Abstract in Korean

새로운 적혈구 생성 촉진제 GC1113의 정맥 또는 피하 투여시의 효과와 약동학적 특성에 대한 탐색 연구

서론: GC1113 은 erythropoietin 에 Fc가 결합된 형태의 새로운 적혈구 생성 촉진제로, 긴 작용시간을 보일 것으로 예상되었다. 전임상실험에서 NESP®(Darbepoetin alfa) 투여시보다 GC1113 투여 후에 hemoglobin 의 증가가 더 오래 지속되는 것을 확인하였다. 이에 본 연구는 사람에서 단회 정맥 또는 피하 투여 시 GC1113의 약력학, 약동학 및 내약성을 탐색하고 이를 NESP® 투여 시와 비교하는 것을 목적으로 하였다.

방법: 용량군 별 무작위배정, 위약 및 진약 대조, 용량 증량 1상 임상시험이 건강자원자 96명을 대상으로 수행되었다. 약동학, 약력학, 내약성 평가를 위한 혈액 샘플은 GC1113 또는 NESP® 투여 직전과 이후 672시간까지 이루어졌으며, 혈중 erythropoietin 농도는 enzyme-linked immunosorbent assay (ELISA) 방법을 사용하여 측정되었다. 약동학적,

약력학적 파라미터는 non-compartmental 방법을 사용하여 구하였다. 면역원성 평가를 포함한 내약성 평가는 입원기간 동안과 연구가 끝날 때까지 이루어졌다.

결과: Reticulocyte count-시간 변화는 GC1113 3-5 μg/kg 정맥 투여시와 NESP® 30 μg가 비슷한 양상을 나타내었다. 피하 투여 시에는 NESP® 투여 시에 비해 GC1113 투여 시 reticulocyte count 는 더 느리게 peak 에 도달하고 늦게 감소하는 경향을 보였다. 약동학적으로는, GC1113 은 NESP® 에 비하여 빠른 제거를 나타내나 피하 투여 시에 더 느린 흡수를 보인다. GC1113은 임상시험을 수행 용량(정맥 투여 0.3-5 μg/kg, 피하 투여 1-8 μg/kg)에서 내약성을 보였으며, 면역원성은 관찰되지 않았다.

결론: GC1113 은 건강 자원자에서 적혈구 생성 효과를 나타내었다. 정맥 투여 GC1113은 NESP®와 비슷한 적혈구 생성 효과를 보였고, 피하 투여 시에는 GC1113 투여 시 reticulocyte count 의 증가가 더 오래 지속되는 양상을 보였다. 임상시험 수행한 용량범위에서 GC1113 은 효과적이고 내약성을 보인다. 이는 추후 환자군을 대상으로 하는 임상시험에 적용 될 수 있을 것이다.

중심단어: 약동학, 약력학, 적혈구 생성, 건강 자원자

학번: 2012-21776

의학석사 학위논문

Exploration of the Effect and Pharmacokinetics of Intravenous and Subcutaneous GC1113, a Novel Erythropoiesis-Stimulating Agent

새로운 적혈구생성 촉진제 GC1113 의 정맥 또는 피하 투여시의 효과와 약동학적 특성에 대한 탐색 연구

2014년 2월

서울대학교 대학원

의학과 협동과정 임상약리학전공

한 혜 경

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지도 교수 신 상 구

이 논문을 의학석사 학위논문으로 제출함

2013년 10월

서울대학교 대학원

의학과 임상약리학 전공

한 혜 경

한혜경의 의학석사 학위논문을 인준함

2013년 12월

위 원 장 _____ 이 동 순 _____ (인)

부위원장 _____ 신 상 구 _____ (인)

위 원 _____ 유 경 상 _____ (인)

Abstract

Exploration of the effect and pharmacokinetics of intravenous and subcutaneous GC1113, a novel erythropoiesis-stimulating agent

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Introduction: GC1113, a hybrid Fc fused erythropoietin, is a novel erythropoiesis-stimulating agent which is expected to have an extended duration of action. The preclinical data showed that the hemoglobin increase lasted longer following GC1113 administration than it did following the administration of

NESP®(Darbepoetin alfa). This study aimed to investigate the pharmacodynamic (PD), pharmacokinetic (PK) characteristics and tolerability profiles of GC1113 in humans after single intravenous (IV) or subcutaneous (SC) administration and to compare the results with those for NESP®.

Methods: A dose-block randomized, placebo- and active- controlled, dose-escalation, phase 1 clinical trial was conducted with 96 healthy volunteers. Blood samples were collected before and up to 672 hours after drug administration and the erythropoietin concentration following the GC1113 or NESP® administration was measured by an enzyme-linked immunosorbent assay (ELISA). PK and PD parameters were determined using noncompartmental methods. Tolerability including immunogenicity evaluation was monitored during hospitalization and until the end of the study.

Results: The reticulocyte count-time profiles in the IV GC1113 3–5 μg/kg groups were comparable with those of the NESP® 30 μg. After subcutaneous administration of GC1113, reticulocyte count peaked later and decreased more slowly than it did following NESP® administration. For pharmacokinetics, GC1113 showed faster elimination and slower absorption through subcutaneous administration than NESP®. The GC1113 (0.3–5 μg/kg for IV, 1–8 μg/kg SC) was well tolerated in the volunteers, and no immunogenicity was observed.

Conclusions: GC1113 showed erythropoietic activity in healthy volunteers. Intravenous GC1113 showed comparable erythropoietic activity to NESP®, and

following subcutaneous administration, the reticulocyte count increase lasted longer for GC1113 than for NESP®. GC1113 was tolerated and effective in the studied dose range; these findings could be applied to further clinical studies with patients.

Keywords: Pharmacokinetics, pharmacodynamics, erythropoiesis, healthy volunteers

Student number: 2012-21776

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List of abbreviations and symbols

AE	Adverse event
AUC	Area under the concentration-time curve
AUC_{inf}	AUC from the drug administration to time infinity
AUC_{last}	AUC from the drug administration to the last measurable concentration
C_{max}	Maximum observed plasma concentration
$\Delta AUEC_{last}$	The area under the baseline corrected pharmacodynamic value-time curve from time 0 to the last measurable value
ΔE_{max}	The maximum baseline corrected pharmacodynamic value
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
EPO	Erythropoietin
IgG	Immunoglobulin G
IV	Intravenous
LLOQ	Lower limit of quantification
PD	Pharmacodynamics
PK	Pharmacokinetics

rHuEPO	Recombinant human erythropoietin
SC	Subcutaneous
SD	Standard deviation
sTfR	soluble transferrin receptor
T_{max}	Observed time of maximum plasma concentration

INTRODUCTION

Erythropoietin (EPO) is a glycoprotein that is mainly produced in the kidneys in response to hypoxia, and it plays a crucial role in producing and maturing erythrocytes [1]. EPO stimulates the maturation of erythrocytes by binding to the EPO receptor on the surfaces of erythroid precursor cells in bone marrow [2]. After gene cloning was succeeded in 1985 [3, 4], recombinant human EPOs (rHuEPOs) were produced and used in clinical settings [5]. rHuEPOs have been used to treat the anemia with chronic kidney disease and cancer chemotherapy [6]. Since the first recombinant EPO (Epoetin alfa) was developed, a variety of long-acting and modified EPO derivatives have been introduced to the market [7]. Darbepoetin alfa (NESP®) is a hyperglycosylated rHuEPO that contains 2 additional carbohydrate chains, resulting in a longer elimination half-life [8]. Mircera®, methoxy polyethylene glycol-epoetin beta, has a prolonged half-life because of PEGylation [9]. In dialysis patients, intravenous (IV) NESP® has a half-life of 25 hours, which is 3 times longer than that of the first-generation rHuEPOs [10]. The half-life of Mircera® was approximately 130 hours with IV or subcutaneous (SC) administration [11].

GC1113 is a novel erythropoiesis-stimulating agent that is under development. GC1113 combines natural EPO with a hybrid Fc using continuous protein antibody fusion technology. Its molecular weight is approximately 113 kDa, which is 3 times

larger than that of NESP® at equipotent molecule. It is known that fusion with Fc portion of immunoglobulin G (IgG) can increase the serum half-life of therapeutic monoclonal antibodies [12]. Based on this finding, GC1113 was developed to increase the serum half-life through fusion of EPO with hybrid Fc portion, consisting of IgG4 and IgD. Through its increased half-life, GC1113 should be more convenient to administer via the IV or SC route for anemic patients.

The preclinical data showed that GC1113 had comparable or longer elimination half-lives, and its effect on erythropoiesis lasted longer than that of NESP®. In normal and acute renal failure-induced rats, both IV and SC GC1113 showed erythrocyte stimulation. The maximum hemoglobin levels of normal rats after IV administration were 16.7 g/dL for GC1113 and 16.3 g/dL for equipotent NESP®. The maximum hemoglobin levels after SC administration were 16.6 g/dL for GC1113 and 15.2 g/dL for equipotent NESP®. In monkeys, GC1113 showed a longer half-life than NESP® for both IV and SC administrations (approximately 1.6-fold for IV and 3-fold for SC). It was well tolerated during toxicology testing in rats and monkeys for single and multiple administrations (unpublished data).

The objectives of this study were to evaluate the pharmacodynamic (PD), pharmacokinetic (PK) and tolerability of GC1113 for both IV and SC administrations in healthy male volunteers. These assessments could help to decide the optimal dosage regimen for later phase clinical trials with anemic patients. In addition, the PD and PK characteristics of GC1113 were compared to those of an

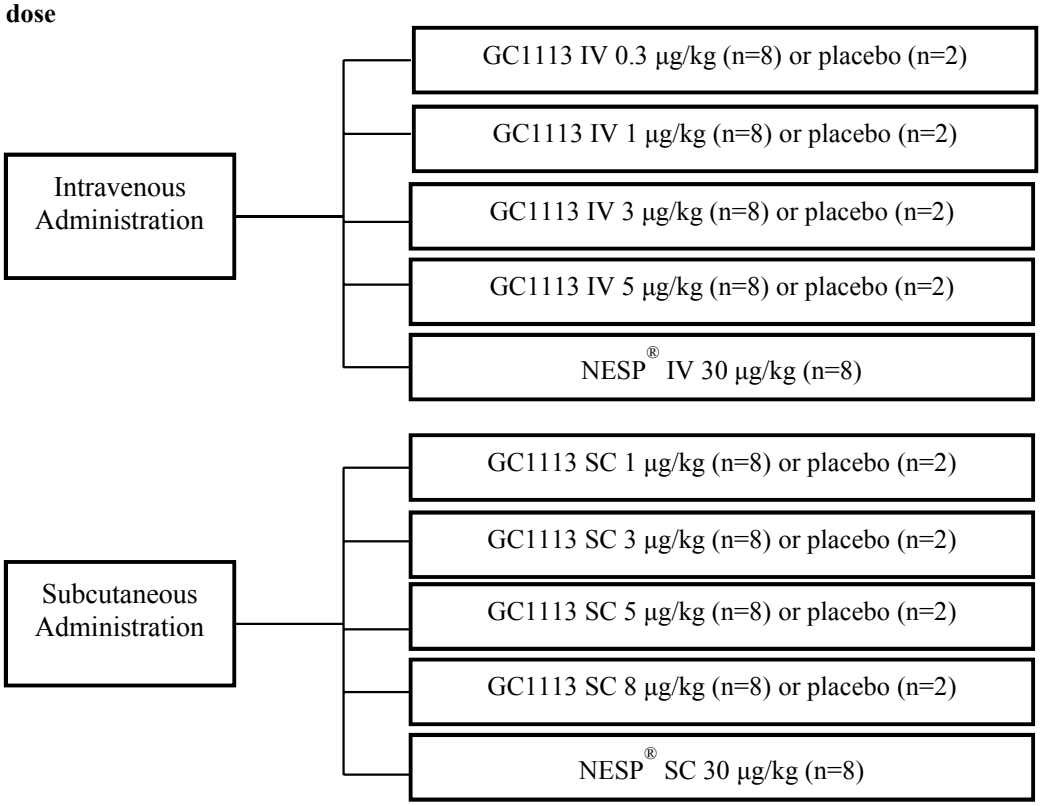
active control, NESP®, for both administration routes.

MATERIALS AND METHODS

Study design

This study was conducted in 2 parts; IV administration and SC administration. In each part, a dose block-randomized, double-blind, single-dose, dose-escalation study was performed for GC1113 and a placebo. An Intravenous 0.3, 1, 3, or 5 μg/kg dose of GC1113 or a 1, 3, 5, or 8 μg/kg dose of SC GC1113 was administered. For the active control in each part, a single dose of 30 μg of NESP® was administered in an open-label manner. The subject disposition data are summarized in Figure 1. The study was conducted at the Clinical Trials Center of Seoul National University Hospital (SNUH) between June 2011 and July 2012. The study protocol was approved by the SNUH Institutional Review Board. All study procedures were performed in accordance with the Declaration of Helsinki and the guidelines for Good Clinical Practice (ClinicalTrials.gov Identifier: NCT01363934).

Figure 1. Subject disposition by route of administration, investigational drugs and its dose



All study participants provided written informed consent before the eligibility screening test was conducted. After the study on each dose group of GC1113 was completed, the interim safety analysis of the prior dose group was performed by an independent data monitoring committee. Iron is needed in erythropoiesis, and iron deficiency can limit erythropoietic activity. To evaluate erythropoietic activity (independent of iron levels), oral iron supplements were provided to subjects with ferritin levels under 100 μg/L during the study period. To evaluate the PK and PD, blood samples were collected before administering the drug and up to day 29 after the drug administration (at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96, 120, 168, 240, 336, 504, and 672 hours for the PK and at 4, 8, 12, 24, 48, 60, 72, 96, 120, 168, 240, 336, 504, and 672 hours for the PD).

Pharmacodynamic assessments

The reticulocyte count, hemoglobin, and reticulocyte-hemoglobin content for each subject were collected for the PD assessments. The reticulocyte-hemoglobin content was obtained by measuring the amount of hemoglobin contained in the reticulocytes to provide an indirect measurement of the functional iron available for erythrocyte formation [13]. In addition, iron store status of each subject was determined to evaluate erythropoiesis by measuring levels of ferritin, soluble transferrin receptor (sTfR), and transferrin saturation.

To more accurately estimate the inter-individual variability, the measured PD values were corrected by the individual baseline PD values, and then a noncompartmental analysis was performed. The maximum baseline corrected PD value (ΔE_{max}) was determined from the observed data. The area under the baseline corrected PD value-time curve from time 0 to the last measurable value ($\Delta AUEC_{last}$) was calculated using the linear trapezoidal summations. To evaluate the time course of the reticulocyte count, the $\Delta AUEC_{last}$ from the time of the drug administration to 2 weeks and 4 weeks after the drug administration were compared.

Determining the erythropoietin concentration

To determine the erythropoietin concentration following the GC1113 or NESP® administration, an enzyme-linked immunosorbent assay (ELISA) using the Human EPO Immunoassay kit (R&D Systems Inc., Minneapolis, MN) was used. The same analytic method was used for all groups throughout the study, but the lower limits of quantification (LLOQs) were different among the groups. The LLOQ of IV administration group was 1.56 ng/mL. To precisely determine the PK profiles of SC, LLOQs were established lower in SC than those of IV. The LLOQs were 156.25 pg/mL for SC GC1113 1, 3, and 5 μg/kg dose groups and 78.125 pg/mL for SC GC1113 8 μg/kg dose group and NESP® 30 μg group. The coefficient of correlation (γ^2) was 0.998 or

greater for this set of analyses.

Pharmacokinetic assessments

The individual serum concentration-time profiles for each subject and the mean concentration values according to the sampling time were plotted. Non-compartmental method was employed to analyze the individual PK parameters using Phoenix® (version 1.2; Pharsight Corporation, Sunnyvale, CA). The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were determined directly from the observed values. The terminal elimination rate constant (λ_z) was calculated by linear regression of the terminal slope of the log-transformed individual plasma concentration-time data. The effective half-life was calculated as ln(2)•MRT, which MRT (mean residence time) is the average amount of time that the drug resides in the body. We calculated the effective half-life because detecting endogenous EPO influence the estimation of λ_z. The area under the concentration-time curve (AUC) from time 0 to the last measurable concentration (AUC_{last}) was calculated using the linear trapezoidal and log- linear trapezoidal summations for the increasing and decreasing phases of the individual plasma concentration-time curve, respectively. The AUC from time 0 to infinity (AUC_{inf}) was calculated using the following formula: $AUC_{inf} = AUC_{last} + C_t/ \lambda_z$, where C_t is the last plasma concentration measured. To evaluate the dose-linearity, the comparisons of

dose-normalized C_{max} and AUC_{last} were performed using Kruskal-Wallis test. Because of its lower LLOQ, endogenous EPO was detected in SC administration group. For the SC group, baseline corrected and dose-normalized PK parameters were used to evaluate the dose-linearity. To evaluate the PK-PD relationship, the PK parameters (C_{max}, AUC_{last}) and PD parameters of the reticulocyte counts (ΔE_{max}, $\Delta AUEC_{last}$) were compared using Pearson's correlation analysis.

Tolerability

Safety profiles were monitored during hospitalization and until the end of the study using laboratory tests (hematology, chemistry, coagulation and urinalysis), vital signs, 12-lead electrocardiograms, spleen sonography, and knee radiography, and the determination of adverse events (AEs). AEs were monitored by asking subjects general health-related questions at the scheduled physical examinations and by self-reporting from the subjects during the study. In addition, for the GC1113 administration group, an immunogenicity evaluation was conducted using ELISA with the GC1113 Ab ELISA Q Kit (BioNote Inc., Korea). Samples for the GC1113 antibody test were collected prior to and at 15 and 29 days after the GC1113 administration.

Results

Pharmacodynamic analysis

A total of 100 healthy Korean subjects received GC1113 (or placebo) or NESP®, and 96 subjects completed the study (1 subject, withdrawal of consent; 1 subject, AE – ear abscess; 2 subjects, lost to follow-up). The baseline corrected reticulocyte count was evaluated as a primary PD parameter. After drug administration with either GC1113 or NESP®, the baseline corrected reticulocyte count increased compared with the placebo group (Figure 2). When the same dose was administered via different administration routes, the $\Delta AUEC_{last}$ of the SC groups increased more than the IV group for both GC1113 and NESP®. GC1113 and NESP® showed similar timing of their reticulocyte count change patterns after the IV administration. The IV GC1113 3–5 μg/kg groups showed similar PD-time profiles compared to the NESP® 30 μg group. After SC administration, the PD time courses of GC1113, including the time to reach the maximum reticulocyte count, were different among the SC dose groups. After 2 weeks from drug administration, the change in the reticulocyte count increased as the dose increased in IV administration. In SC administration, little difference was observed among the dose groups, and great variability was observed 4 weeks after drug the administration (Figure 3).

There was no dose-related trend in the hemoglobin, reticulocyte-hemoglobin content, and transferrin saturation values (Figure 4). Ferritin appeared to decrease and return to baseline, and sTfR appeared to increase and then return to baseline, both dependent on the dose administered (Figure 5, 6).

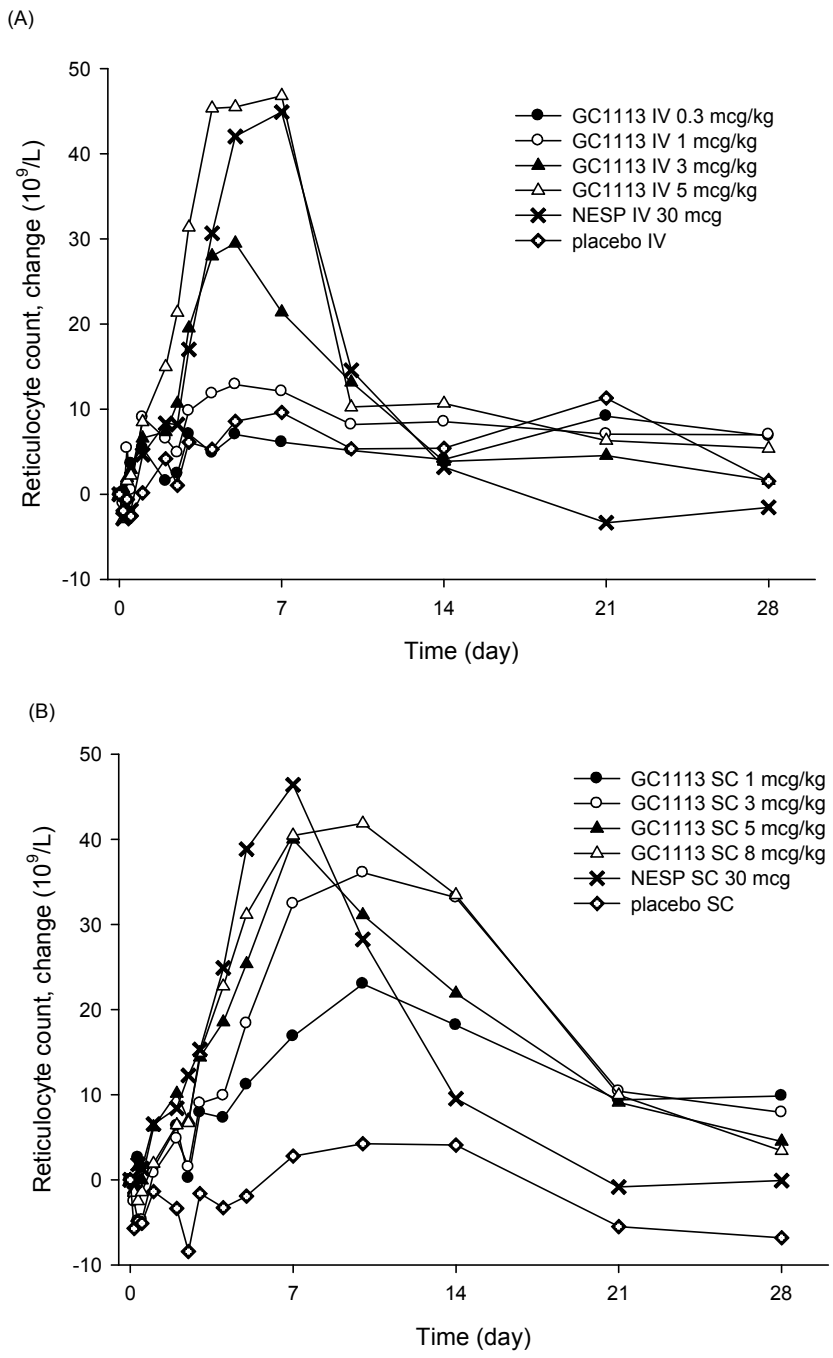


Figure 2. Mean reticulocyte count change from baseline–time profiles up to 672 hours (Day 29) after a single administration of GC1113, NESP® or placebo. (A) intravenous administration, (B) subcutaneous administration

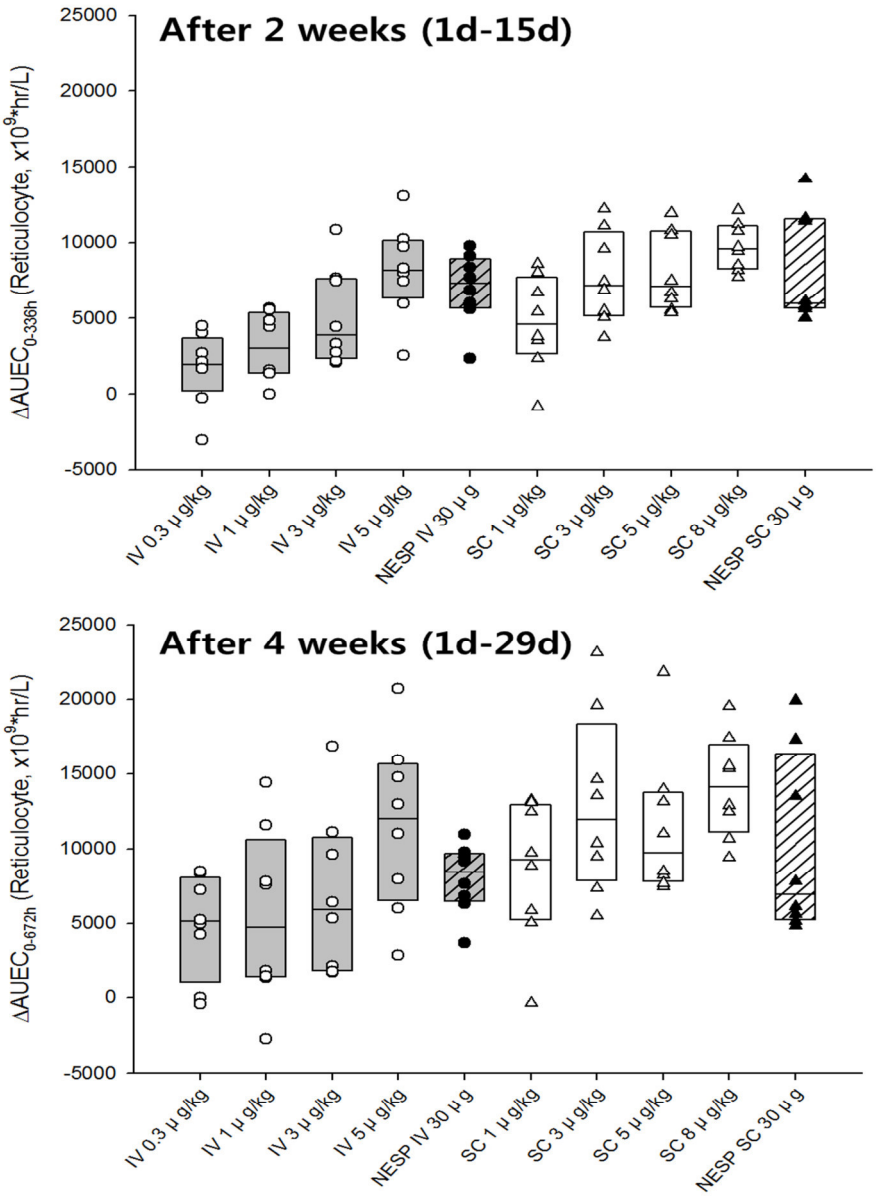


Figure 3. Individual baseline corrected AUECs (area under the reticulocyte count value -time curve) up to 2 weeks and 4 weeks after a single administration of GC1113 or NESP® (GC1113 IV 0.3, 1, 3, 5 μg/kg, NESP® IV 30 μg, GC1113 SC 1, 3, 5, 8 μg/kg, NESP® SC 30 μg)

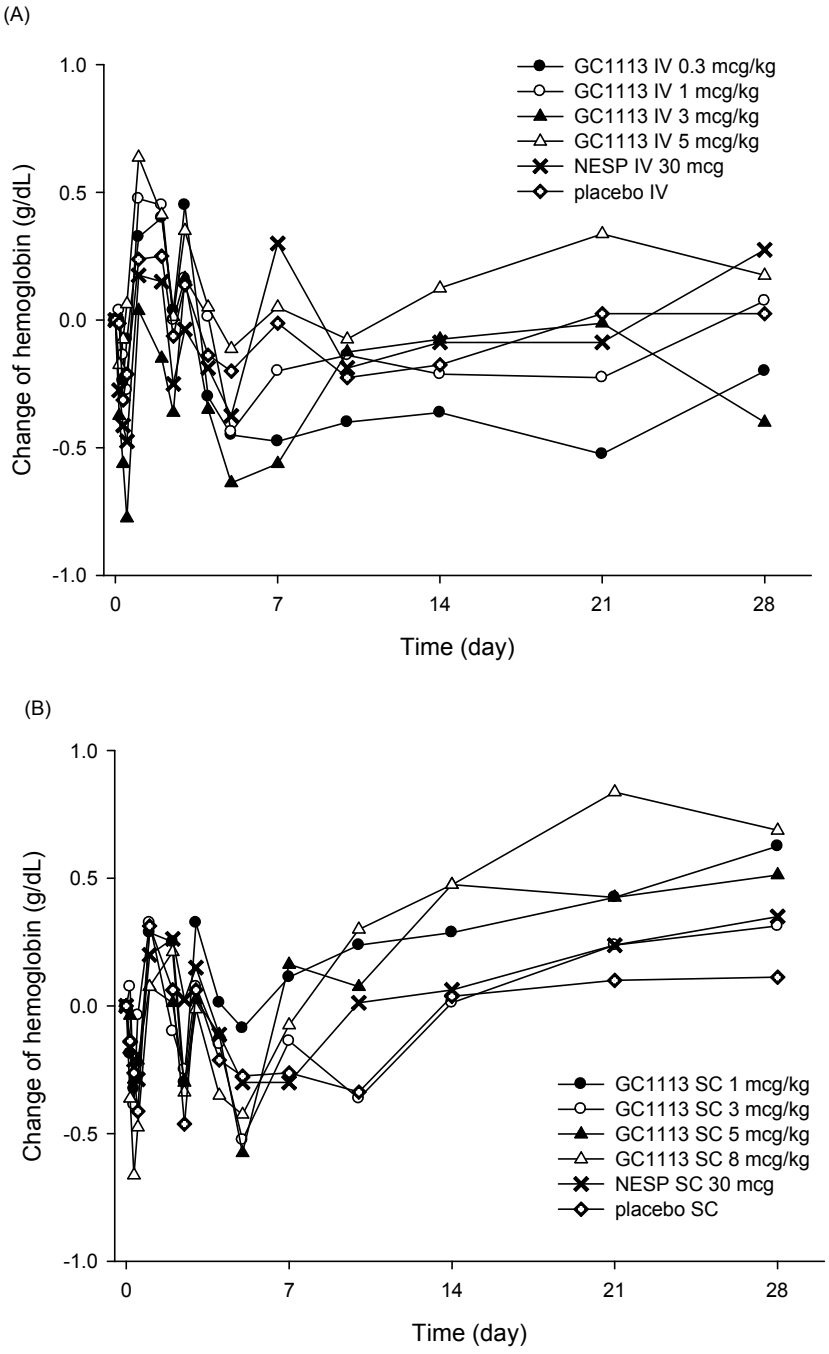


Figure 4. Mean hemoglobin change from baseline–time profiles up to 672 hours (Day 29) after a single administration of GC1113 or NESP® or placebo (A: intravenous administration, B: subcutaneous administration)

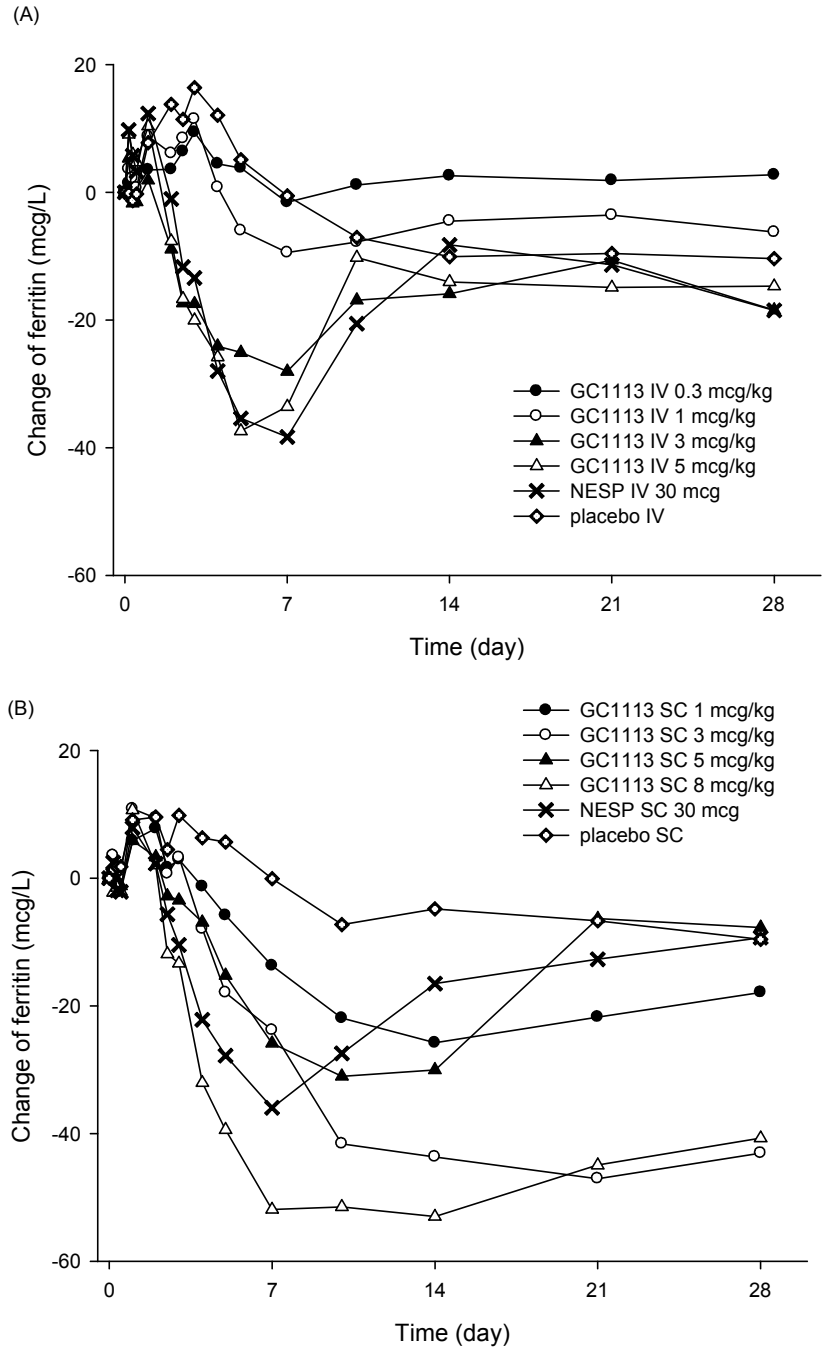


Figure 5. Mean ferritin change from baseline–time profiles up to 672 hours (Day 29) after a single administration of GC1113 or NESP® or placebo (A: intravenous administration, B: subcutaneous administration)

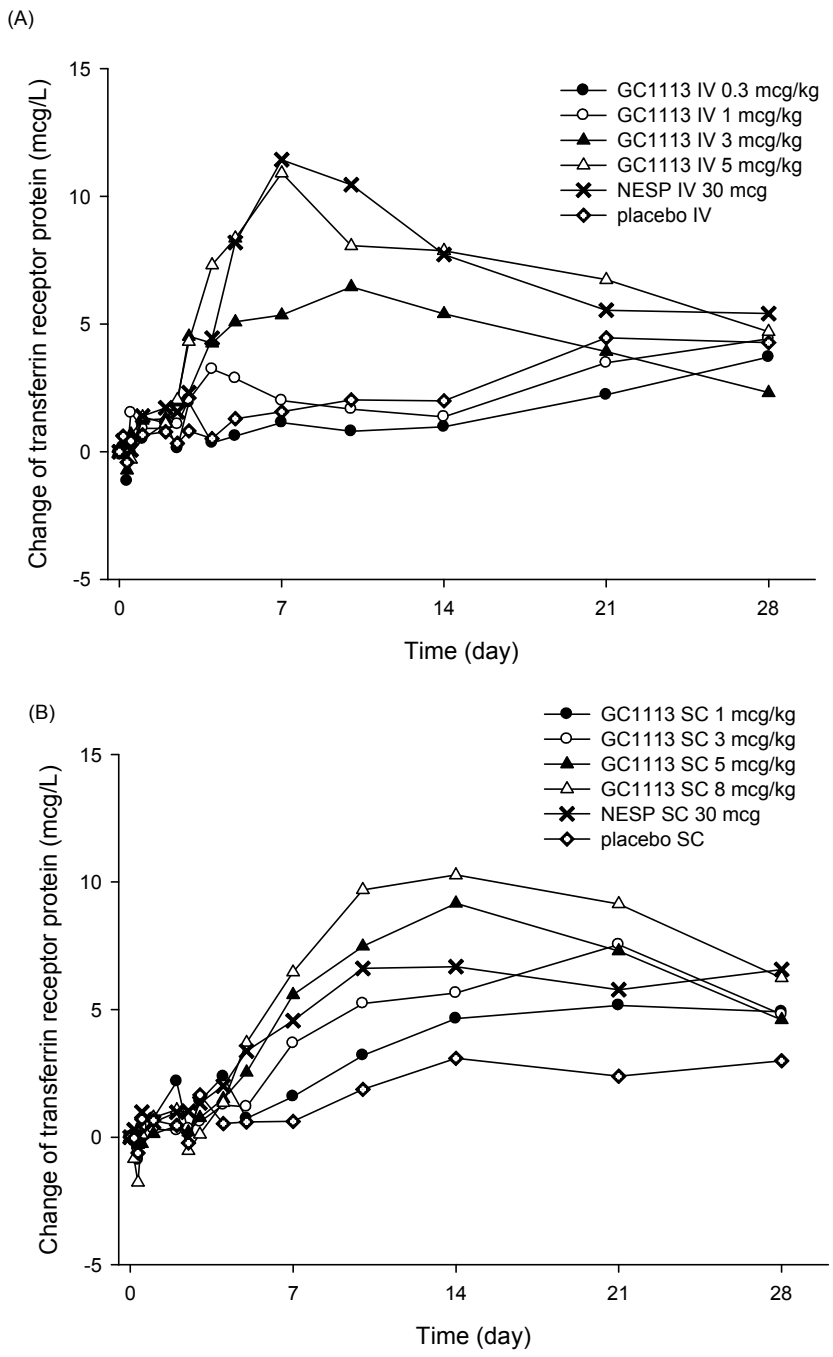


Figure 6. Mean transferrin receptor protein change from baseline –time profiles up to 672 hours (Day 29) after a single administration of GC1113 or NESP® or placebo (A: intravenous administration, B: subcutaneous administration)

Pharmacokinetic analysis

The C_{max} and AUC_{last} of IV NESP® 30 μg were closest to those of IV GC1113 at 1 μg/kg, but the effective half-life was longer for IV NESP® 30 μg than for IV GC1113 at 1 μg/kg. The effective half-life of NESP® 30 μg was similar to that of IV GC1113 3–5 μg/kg (Figure 7, Table 1, 2). All SC GC1113 (1–8 μg/kg) groups and SC NESP® 30 μg group showed similar effective half-lives. Based on the Kruskal-Wallis test results, the dose-normalized C_{max} indicated that C_{max} increased proportionally with increasing dose ($P = 0.264$); however, the dose-normalized AUC_{last} values were different among the dose groups for IV administration ($P < 0.001$) (Figure 8). For the SC group, baseline corrected and dose-normalized C_{max} and AUC_{last} did not differ from the dose groups ($P = 0.730$ for C_{max}, $P = 0.102$ for AUC_{last}) (Figure 9).

Table 1. Summary of pharmacokinetic and pharmacodynamic parameters following intravenous administration of GC1113 or NESP®

	Intravenous administration				
Drug	GC1113				NESP®
Dose	0.3 μg/kg (n=8)	1 μg/kg (n=8)	3 μg/kg (n=8)	5 μg/kg (n=8)	30 μg (n=8)
Pharmacokinetic parameters					
C_{max} (μg/L)	4.82 (1.4)	18.99 (3.47)	51.41 (16.8)	88.09 (15.33)	12.17 (1.71)
AUC_{last} (hr•μg/L)	21.32 (12.07)	147.61 (57.76)	826.32 (342.93)	1759.72 (315.09)	210.16 (35.36)
AUC_{inf} (hr•μg/L)	39.82 (10.78)	191.33 (59.51)	877.60 (323.03)	1799.65 (313.28)	264.75 (38.78)
Effective half-life (hr)	1.99 (0.89)	3.87 (1.02)	7.98 (1.92)	10.51 (1.34)	9.29 (0.86)
Pharmacodynamic parameter					
$\Delta AUEC_{last}$ (10^9•hr/L)	4781.60 (3452.15)	5423.35 (5853.46)	6879.04 (5370.52)	11540.14 (5769.97)	7986.48 (2340.06)

Data are presented as mean (standard deviation)

C_{max} (maximum concentration), AUC (Area under the concentration-time curve), AUC_{last} (AUC from time 0 to the last measurable concentration), AUC_{inf} (The AUC from time 0 to infinity), $AUEC_{last}$ (Area under the baseline-corrected reticulocyte count -time curve)

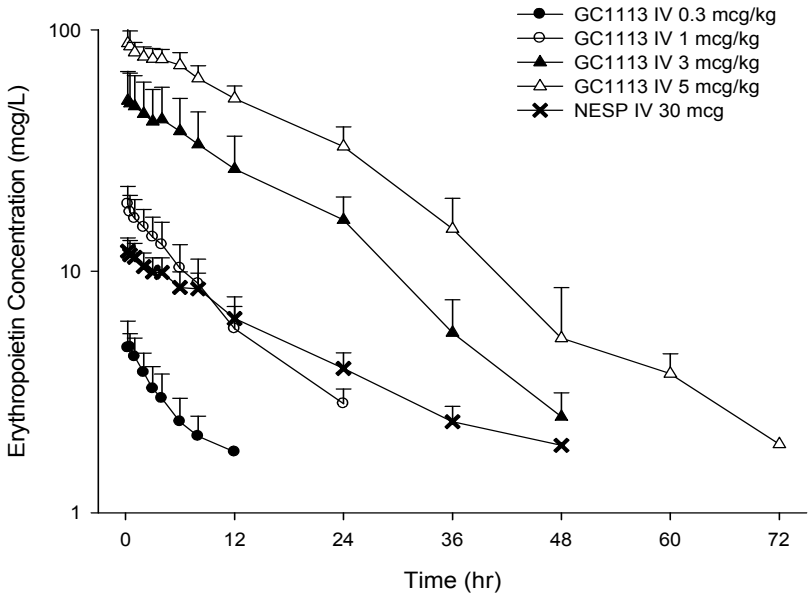
Table 2. Summary of pharmacokinetic and pharmacodynamic parameters following subcutaneous administration of GC1113 or NESP®

	Subcutaneous administration				
Drug	GC1113				NESP®
Dose	1 μg/kg (n=8)	3 μg/kg (n=8)	5 μg/kg (n=8)	8 μg/kg (n=8)	30 μg (n=8)
Pharmacokinetic parameters					
C_{max} (μg/L)	0.87 (0.45)	1.72 (1.26)	2.81 (1.88)	5.71 (4.8)	1.07 (0.34)
AUC_{last} (hr•μg/L)	198.46 (106.27)	287.46 (102.65)	443.24 (73.23)	615.97 (147.05)	216.48 (52.47)
AUC_{inf} (hr•μg/L)	341.74 (179.32)	374.97 (83.07)	514.52 (54.12)	716.07 (136.88)	315.00 (101.58)
Effective half-life (hr)	157.88 (33.13)	151.58 (35.79)	145.89 (37.3)	138.45 (36.43)	157.33 (13.09)
Pharmacodynamic parameter					
$\Delta AUEC_{last}$ (10^9•hr/L)	8480.03 (4740.09)	12935.85 (6037.02)	11480.05 (4863.71)	14140.63 (3412.19)	9983.65 (5940.2)

Data are presented as mean (standard deviation)

C_{max} (maximum concentration), AUC (Area under the concentration-time curve), AUC_{last} (AUC from time 0 to the last measurable concentration), AUC_{inf} (The AUC from time 0 to infinity), $AUEC_{last}$ (Area under the baseline-corrected reticulocyte count -time curve)

(A)


GC1113 IV 0.3 mcg/kg
GC1113 IV 1 mcg/kg
GC1113 IV 3 mcg/kg
GC1113 IV 5 mcg/kg
NESP IV 30 mcg
Erythropoietin Concentration (mcg/L)
100
10
1
0
12
24
36
48
60
72
Time (hr)

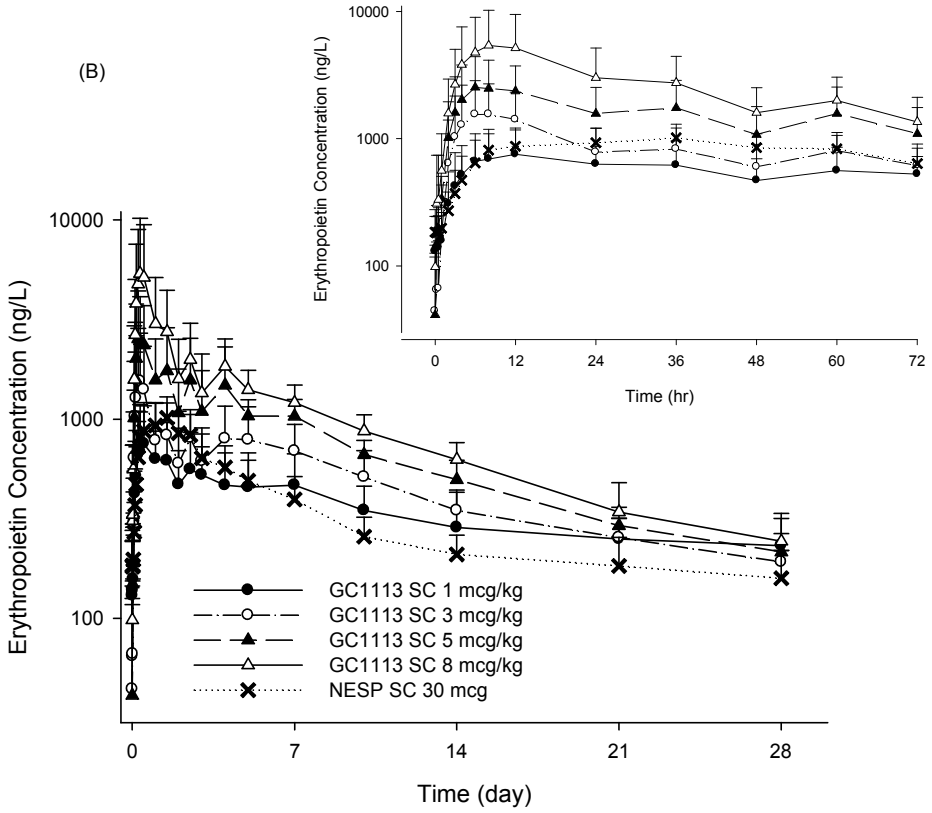


Figure 7. Mean serum concentration-time profiles of GC1113 or NESP® after single (A) intravenous and (B) subcutaneous administration of 0.3, 1, 3, 5 µg/kg of GC1113 or 30 µg of NESP®. Inset shows the initial 72 hours portion of the profile. (log-linear scale)

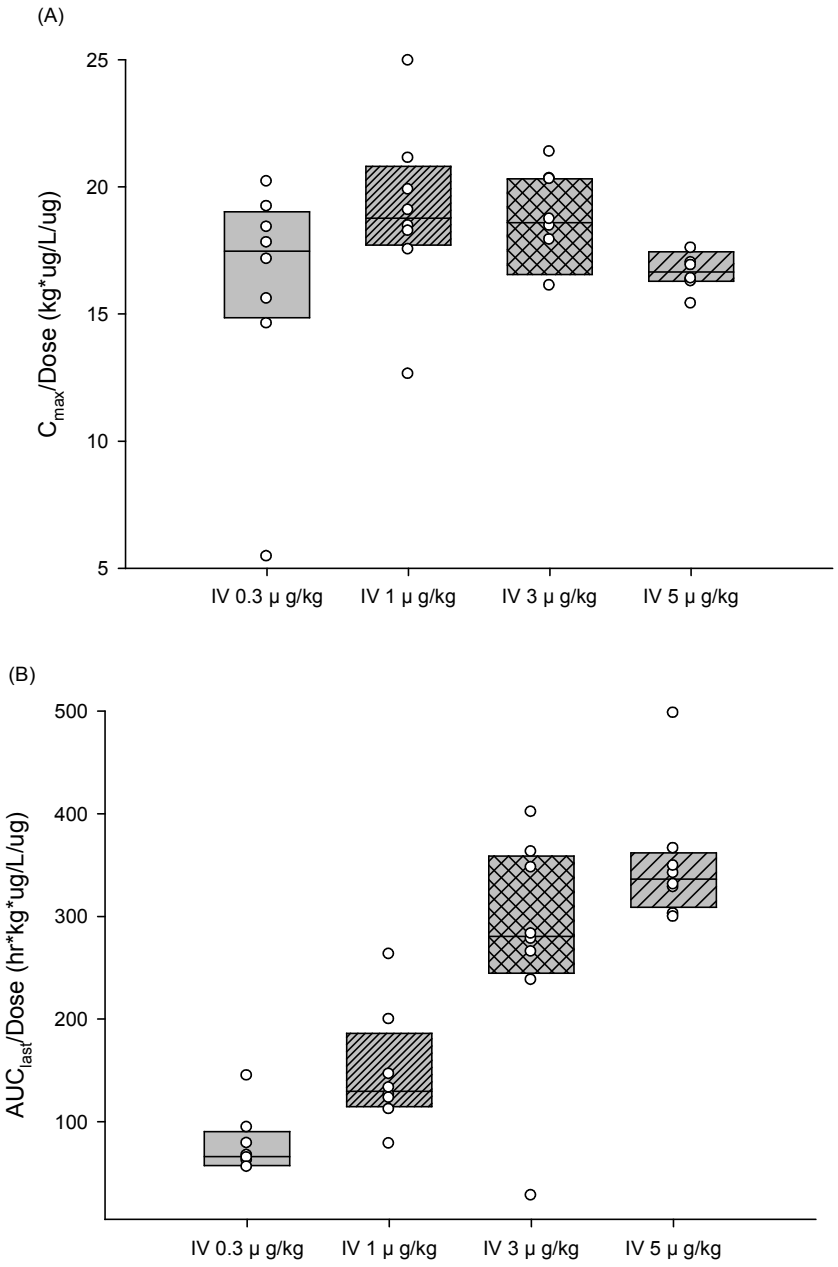


Figure 8. Individual Dose-normalized C_{max} and AUC_{last} values by intravenous GC1113 treatment. Open circle represents the individual values. Box plot provides median and 25%/75% quartiles.

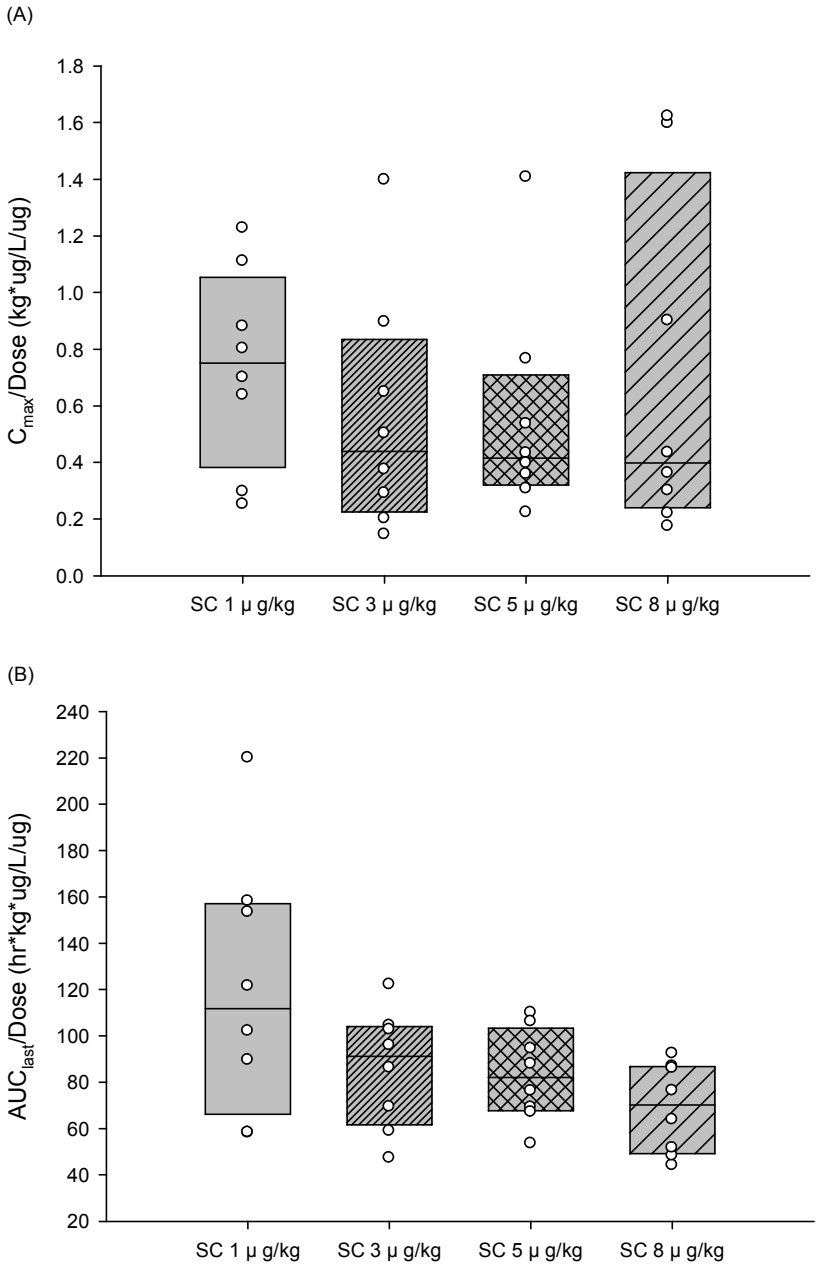


Figure 9. Individual Dose-normalized, baseline corrected C_{max} and AUC_{last} values by subcutaneous GC1113 treatment. Open circle represents the individual values. Box plot provides median and 25%/75% quartiles.

Investigating the relationship between the PK parameters (C_{max}, AUC_{last}) and PD parameters (reticulocyte count ΔE_{max}, $\Delta AUEC_{last}$) using Pearson's correlation analysis, the IV groups showed a positive and statistically significant (*P*-value < 0.05) correlation between the PK and PD parameters. However, the SC groups did not demonstrate linearity between their PK and PD parameters (Figure 10).

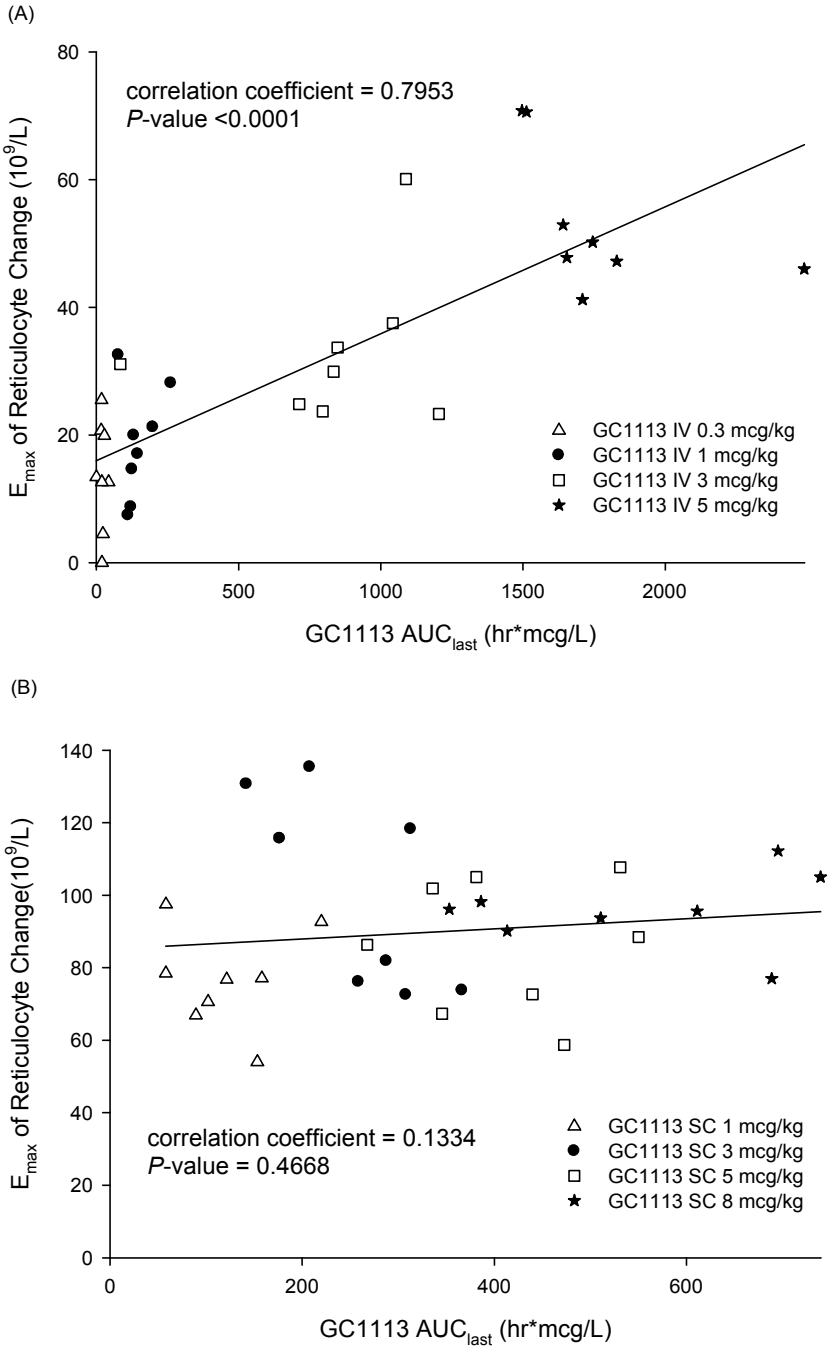


Figure 10. Pharmacokinetic-pharmacodynamic relationship of AUC_{last} (area under the time-concentration curve from time 0 to last measurable concentration) vs E_{max} (maximum observed reticulocyte) of baseline corrected reticulocyte after

single GC1113 administration. (A) intravenous administration, (B) subcutaneous administration

Tolerability

Among the 100 subjects, 39 experienced 70 adverse events. Among the adverse events that were related to GC1113, headache was the most frequently reported (6 cases), followed by 3 cases each of rhinorrhea and upper respiratory tract infection. All adverse events were mild or moderate in intensity, and the subjects recovered. There was no serious adverse event. In addition, there were no clinically significant changes in the laboratory tests, vital signs, ECGs, spleen sonography, or knee radiography. Iron was provided as needed to the 96 subjects with ferritin levels under 100 μg/L during the clinical trial. No GC1113 antibody formation was detected throughout the study period.

Discussion

This study explored the effectiveness and tolerability of GC1113 in healthy volunteers. GC1113 showed erythropoietic activity based on not only in the reticulocyte count increment but also on other PD and iron parameters, such as ferritin and sTfR. Because this study was the first to observe GC1113 administration in humans, the safety profiles were intensively monitored during the study. No immunogenicity was found after a single administration in this study as reported for other Fc fusion drugs [14].

The reticulocyte count increased after the IV and SC administration of GC1113. The IV formulation of 3–5 μg/kg GC1113 showed PD profiles similar to those of 30 μg of NESP®. After the SC administration of GC1113, the reticulocyte count level peaked later and decreased more slowly compared with the administration of NESP®. The SC GC1113 is expected to have longer dosing interval than NESP®. Considering reticulocyte count-time profile (Figure 2), reticulocyte counts returned to its baseline value after 2 weeks in IV administration and it took 4 weeks to return baseline value in SC administration. For IV administration, the dose-response relationship was observed much obviously after 2 weeks than 4 weeks from drug administration (Figure 3). For SC administration, the dose-response relationship can be determined after 4 weeks from drug administration. After

4 weeks, the $\Delta AUEC_{last}$ increased sub-proportionally to the dose increase and the great inter-individual variability is observed both SC GC1113 and NESP®. Further evaluations in anemic patients are needed to confirm the dose-response of GC1113.

When considering the PD parameters all together including reticulocytes, GC1113 increased the erythropoietic activity in humans and is expected to be effective in anemic patients. In addition to the reticulocyte count change, the ferritin level was decreased, and the sTfR was increased after the GC1113 and NESP® administration, and then it returned to baseline levels. Ferritin reflects the status of iron, which is required to form erythrocytes [15]. Increased sTfR levels indicate increased erythropoietic activity or tissue iron deficiency [16]. During the study, the subjects' iron levels were monitored and supplemented to prevent deficiency. Thus, the sTfR increase in this study represented erythropoietic activity.

In terms of PK, the effective half-life of GC1113 was slightly shorter than NESP® when delivered via IV and longer than NESP® when delivered SC. The terminal slope from the concentration-time profile of GC1113 was steeper for IV administration and flatter for SC administration compared with NESP®. After SC administration, the drug absorption continued even in the elimination phase, and the SC elimination phase was presumably affected by not only the elimination but also the slow absorption rate [17]. This can explain why the effective half-life was longer after SC administration, despite

GC1113's elimination by a common pathway regardless of the administration route. In addition, SC GC1113 showed slower absorption than SC NESP®. The molecular size of GC1113 is 3 times larger than NESP®, which could be the reason for the slower absorption following the SC administration of GC1113 compared with NESP®. There may also be some relationship between the existence of the Fc portion and the SC absorption rate; however the effect of the Fc portion on SC bioavailability is not yet known [18].

The elimination mechanism of EPO has not been fully elucidated to date, but it is known that EPO receptor mediated endocytosis degradation is a main elimination pathway [19, 20]. Because of the saturation of the receptor-mediated endocytosis elimination pathway, the PK of GC1113, which is an EPO derivative, was expected to be nonlinear. IV GC1113 was judged to have nonlinear PK characteristics based on the Kruskal-Wallis test results using the dose-normalized AUC_{last} and the increase in the effective half-life with the increased dose. For IV administration, the AUC_{last} increased supra-proportionally to the dose increase. However, the dose-normalized C_{max} and AUC_{last} of SC GC1113 were not different among the 1–8 μg/kg dose groups. Because the SC elimination phase was affected by slow absorption, C_{max} and AUC_{last} were less affected by saturation of the receptor-mediated endocytosis pathway. Considering the PK and PD parameters together, a positive correlation was observed, particularly in the relationship between the AUC_{last} and ΔE_{max} in the IV administration group. There was no obvious correlation

between the PK and PD in the SC administration group (Figure 10). The time delay between the EPO concentration and the response in the erythrocyte counts is known[21], and this relationship was also observed in this study. We evaluated only the linear relationship of the PK and PD parameters. Further evaluation using a time delay model to consider an indirect PK-PD relationship would be helpful to understand the relationship between PK and PD.

The terminal half-life of NESP® in this study was shorter than that in other published studies; in previous reports on renal disease or cancer patients, the terminal half-life of NESP® was approximately 25-33 hours following IV administration and approximately 49–105 hours following SC administration [10, 22-24]. The terminal half-life of NESP® in this study (mean ± standard deviation) was 13.0 ± 4.7 hours for IV administration and 79.7 ± 38.3 hours for SC administration. Chemotherapy can reduce the clearance of NESP®[25], because bone marrow contributes to the mechanism of cell-mediated clearance of erythropoietin [26]. Thus, patients undergoing chemotherapy might show longer half-lives than healthy volunteers.

There were some limitations to making direct comparisons of the PK characteristics of GC1113 and NESP®. It is not known whether the Fc portion of GC1113 separates after administration. It was also difficult to separately quantify the endogenous and exogenous EPO levels within the total measured EPO. These factors impeded direct comparisons of the PK characteristics

between GC1113 and NESP®. However, the increased reticulocyte count could be compared between GC1113 and NESP®, and GC1113 showed comparable efficacy to NESP®.

This study evaluated the PD, PK and tolerability of a novel erythropoiesis-stimulating agent using IV and SC formulations. IV GC1113 showed comparable erythropoietic activity to NESP®, and following SC administration, the reticulocyte count increase lasted longer for GC1113 than for NESP®. GC1113 was well tolerated, and it was effective in the studied dose range. These results could be applied to further clinical studies in anemia patients with chronic kidney disease and in cancer patients undergoing chemotherapy.

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Abstract in Korean

새로운 적혈구 생성 촉진제 GC1113의 정맥 또는 피하 투여시의 효과와 약동학적 특성에 대한 탐색 연구

서론: GC1113 은 erythropoietin 에 Fc가 결합된 형태의 새로운 적혈구 생성 촉진제로, 긴 작용시간을 보일 것으로 예상되었다. 전임상실험에서 NESP®(Darbepoetin alfa) 투여시보다 GC1113 투여 후에 hemoglobin 의 증가가 더 오래 지속되는 것을 확인하였다. 이에 본 연구는 사람에서 단회 정맥 또는 피하 투여 시 GC1113의 약력학, 약동학 및 내약성을 탐색하고 이를 NESP® 투여 시와 비교하는 것을 목적으로 하였다.

방법: 용량군 별 무작위배정, 위약 및 진약 대조, 용량 증량 1상 임상시험이 건강자원자 96명을 대상으로 수행되었다. 약동학, 약력학, 내약성 평가를 위한 혈액 샘플은 GC1113 또는 NESP® 투여 직전과 이후 672시간까지 이루어졌으며, 혈중 erythropoietin 농도는 enzyme-linked immunosorbent assay (ELISA) 방법을 사용하여 측정되었다. 약동학적,

약력학적 파라미터는 non-compartmental 방법을 사용하여 구하였다. 면역원성 평가를 포함한 내약성 평가는 입원기간 동안과 연구가 끝날 때까지 이루어졌다.

결과: Reticulocyte count-시간 변화는 GC1113 3-5 μg/kg 정맥 투여시와 NESP® 30 μg가 비슷한 양상을 나타내었다. 피하 투여 시에는 NESP® 투여 시에 비해 GC1113 투여 시 reticulocyte count 는 더 느리게 peak 에 도달하고 늦게 감소하는 경향을 보였다. 약동학적으로는, GC1113 은 NESP® 에 비하여 빠른 제거를 나타내나 피하 투여 시에 더 느린 흡수를 보인다. GC1113은 임상시험을 수행 용량(정맥 투여 0.3-5 μg/kg, 피하 투여 1-8 μg/kg)에서 내약성을 보였으며, 면역원성은 관찰되지 않았다.

결론: GC1113 은 건강 자원자에서 적혈구 생성 효과를 나타내었다. 정맥 투여 GC1113은 NESP®와 비슷한 적혈구 생성 효과를 보였고, 피하 투여 시에는 GC1113 투여 시 reticulocyte count 의 증가가 더 오래 지속되는 양상을 보였다. 임상시험 수행한 용량범위에서 GC1113 은 효과적이고 내약성을 보인다. 이는 추후 환자군을 대상으로 하는 임상시험에 적용 될 수 있을 것이다.

중심단어: 약동학, 약력학, 적혈구 생성, 건강 자원자

학번: 2012-21776