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Original Research Article

Comparison of the pharmacokinetic and pharmacodynamic properties of two recombinant granulocyte colony-stimulating factor formulations after single subcutaneous administration to healthy volunteers

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ABSTRACT

Background and objective: The aim of this randomized, single dose, two-period crossover study with two weeks wash-out period was the demonstration of bioequivalence of two recombinant human granulocyte colony-stimulating factor (rG-CSF) formulations after subcutaneous administration of 300 µg comparing their pharmacokinetic (primary endpoints AUC_{0-24} , $AUC_{0-\infty}$ and C_{max}) and pharmacodynamic (primary endpoints ANC AUC_{0-72} , ANC $AUC_{0-\infty}$ and ANC $_{max}$) profiles in healthy male subjects.

Materials and methods: A total of 36 (23.0 ± 6.0 years, 76.6 ± 7.2 kg) healthy subjects were recruited. Using a 1:1 randomization ratio, subjects were randomly assigned to one of two possible treatment-sequence groups to receive the single dose of test formulation (Gp-02) and reference product (NeupogenTM) concentrations were measured by enzyme-linked immunosorbent assay (ELISA) up to 24 h and the Absolute Neutrophil Count (ANC) was determined using hematology analyzer Coulter STKSTM (Beckman Coulter) up to 72 h after injection. The geometric mean of primary pharmacokinetic and pharmacodynamic variables were considered bioequivalent if the 90% confidence intervals (CI) would fall in the bioequivalence range of 80%–125%.

Results: AUC_{0-24} (ratio of means 103.4, 90% CI: 95.6–111.9), $AUC_{0-\infty}$ (103.4, 90% CI: 95.7–111.7), C_{max} (99.6, 90% CI: 89.0–111.4), ANC AUC_{0-72} (100.0, 90% CI: 96.6–103.5), ANC $AUC_{0-\infty}$ (100.8,

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90% CI: 96.5–105.3), and ANC_{max} (100.2, 90% CI: 95.4–105.1) were determined. Single doses of test and reference formulations were well tolerated. The incidence of AEs was equally distributed across treatment groups with the most frequent AEs being headache, fever, and back pain.

Conclusions: The study results demonstrated the bioequivalence of Gp-02, a new formulation of filgrastim, and the reference product Neupogen™.

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1. Introduction

Human granulocyte colony stimulating factor (G-CSF) is a glycoprotein, which regulates the differentiation of neutrophils from progenitor cells and stimulates the release from the bone marrow and also activates circulating neutrophils. Endogenous G-CSF is produced mainly by mononuclear cells and fibroblasts. G-CSF markedly increases neutrophil counts in peripheral blood, slightly elevates levels of monocytes and lymphocytes and mobilizes blood progenitor cells (CD34+) into circulation [1]. Peak levels of neutrophils are reached approximately 12 h after filgrastim administration. Absolute neutrophil count (ANC) returns to pre-treatment levels around 48–72 h after cessation of filgrastim administration. Evaluation of pharmacokinetics in human subjects indicates that recombinant G-CSF (filgrastim) is quickly absorbed after subcutaneous bolus injection and follows first order elimination kinetics. Serum elimination half-life of filgrastim is approximately 3.5 h, with the clearance rate of approximately 0.6 mL/min/kg [2].

New recombinant G-CSF (Gp-02) has been developed by the Sponsor of the study – Sicor Biotech UAB (Teva Group, Lithuania). The recombinant G-CSF is bacterially synthesized, i.e., produced in genetically modified *Escherichia coli*. It differs from endogenous G-CSF since it has an N-terminal methionine residue and is not glycosylated, but the biological activity of recombinant G-CSF is the same as of endogenous human G-CSF. The reference product Neupogen™ (Amgen, Thousand Oaks, USA) was authorized in European Union (EU) more than two decades ago and is used to reduce the duration of neutropenia and the incidence of febrile neutropenia, associated with cytotoxic chemotherapy and also to increase the number of hematopoietic stem cells in the blood before collection by leukapheresis for use in hematopoietic stem cell transplantation.

The physicochemical comparison of two filgrastim products demonstrated similarity regarding molecular weight, amino acid sequence, tertiary structure, impurity profile, as requested by European Medicines Agency (EMA) regulations for biosimilar medicinal products [3]. Current study was designed to collect pharmacokinetic and pharmacodynamic profile of new filgrastim product in support of biosimilarity. The primary objective of the study was the demonstration of bioequivalence of the two filgrastim formulations after subcutaneous administration of 300 µg single dose, comparing

their pharmacokinetic and pharmacodynamic properties in healthy subjects.

2. Materials and methods

2.1. Subjects

The study protocol and amendments were approved by the local Ethics Committee and Lithuanian State Medicines Control Agency (SMCA). The study was conducted in compliance with the Declaration of Helsinki and according to Good Clinical Practice (GCP). All healthy male volunteers who gave their written informed consent to participate and corresponded to selection criteria were included in this study. Volunteers were free to withdraw from the study at any time. Volunteers were considered healthy if they had no history of any chronic diseases and no pathological symptoms or signs at the physical examination and the laboratory test (complete profile of blood cell counts, routine serum biochemistry, urinalysis, hepatitis B surface antigen, hepatitis C antibody and human immunodeficiency virus antibodies). The subjects were not included if they had allergic or idiosyncratic reactions to any drug, any clinically relevant allergic disease, received treatment with blood cell colony-stimulating factors, interleukins or interferons or had general anesthesia or blood donations within 3 months. The subjects were instructed not to take other medications or alcohol throughout the study and to avoid strenuous exertion. Withdrawn subjects were not replaced. The follow-up of withdrawn subjects was carried-out within 14 days after the last study drug administration or when any clinically significant changes were resolved and when the investigator deemed that no further investigations were indicated.

2.2. Study design and sample size

This was a randomized, single dose, two-period crossover study in healthy subjects. There was a two weeks wash-out period after the first treatment period.

The sample size was determined according multiplicative model [4]. A total of 36 healthy subjects were planned to recruit ensuring 80% statistical power ($\alpha = 0.05$) to demonstrate bioequivalence between test formulation Gp-02 and Neupogen™ assuming an intrasubject coefficient of variation (CV) of 21% and a bioequivalence range of 0.80–1.25 for the test (T) and reference (R) area under curve (AUC) mean ratio.

2.3. Study formulations

Test formulation (Gp-02), containing 300 µg filgrastim at a fill volume of 1.0 mL, was manufactured by Sicor Biotech UAB (Teva Group) as sterile solution in pre-filled syringes ready for injection. Reference preparation was the marketed formulation of Neupogen™ containing 300 µg filgrastim at a fill volume of 1.0 mL produced by Amgen Inc (Thousand Oaks, CA, USA), as sterile solution for injection in vials. The Gp-02 formulation had the same strength and composition as the reference product, the marketed medicinal product Neupogen™.

Each eligible subject received a single dose of 300 µg of each: test and reference formulations in a randomized crossover design. Randomization was made using procedure PLAN of SAS. Using a 1:1 randomization ratio, subjects were randomly assigned to one of two possible treatment-sequence groups to receive either T or R drug in Period 1. After a two weeks wash-out period subjects received the alternative treatment in Period 2. The exact dose of 300 µg was administered via the subcutaneous route. The investigational product was administered to the subjects after overnight fasting at around 8.00 A.M. in the back of upper arm.

2.4. Study assessments and analytical methods

Blood samples for serum G-CSF concentration determination were collected by venipuncture into labeled tubes (SST) of 6 mL volume at the following time points: 0, 20, 40, 60, 90 min, 2, 3, 4, 6, 8, 12, and 24 h after injection. G-CSF concentrations were measured by a validated quantitative sandwich enzyme-linked immunosorbent assay (ELISA) method (Quantikine®, R&D System, Inc) at the qualified clinical laboratory (Centre for Communicable Diseases and AIDS, Vilnius, Lithuania). The LLOQ (Lower Limit of Quantification) was 50 pg/mL, the ULOQ (Upper Limit of Quantification) was 1800 pg/mL. Intra-assay CV was 2.8, 1.7 and 1.1% for 280, 827 and 1696 pg/mL, respectively. Inter-assay CVs were 4.1, 3.2 and 3.8% for 176, 1094 and 2169 pg/mL, respectively.

Blood samples for Absolute Neutrophil Count (ANC) were collected by venipuncture at the following time points: 0, 20, 40, 60, 90 min, 2, 3, 4, 6, 8, 10, 12, 16, 24, 32, 40, 48 and 72 h after injection. The ANC was determined using the differential automated hematology analyzer Coulter STKS™ (Beckman Coulter, Inc., Fullerton, CA, USA) at the qualified clinical hematology laboratory (Kaunas Clinical Hospital, Kaunas, Lithuania).

All subjects were analyzed for safety. Standard safety variables were used in the study: vital signs (blood pressure, heart rate, body temperature), laboratory controls: hematology, biochemistry (potassium, creatinine, uric acid), liver function tests (aspartate transaminase (ALT), alkaline phosphatase (AST)), coagulation tests and urinalysis. The subjects were routinely followed until 72 h after injection in both treatment periods. All adverse events (AEs) were followed until their resolution.

2.5. Statistical analysis

The area under the serum concentration-time curve (AUC) from 0 to 24 h and the area under the ANC-time curve from 0 to

72 h were calculated using the trapezoidal rule using the actual times of measurements. The time at which peak concentration/ANC value occurred (T_{max}) was determined directly from the experimental data as the time of maximum concentration/ANC observed (C_{max}) considering the entire curve. The terminal elimination rate constant (λ_z) was calculated from the slope of the semilogarithmic plot of the terminal phase of the plasma concentration-time curve determined by linear regression. The elimination half-life ($T_{1/2}$) was calculated from the terminal elimination rate constant (λ_z). Pharmacokinetic (AUC_{0-24} , $AUC_{0-\infty}$, C_{max} , T_{max} , $T_{1/2}$, λ_z) and pharmacodynamic (ANC AUC_{0-72} , ANC $AUC_{0-\infty}$, ANC $_{max}$, ANC t_{max}) variables were analyzed by non-compartmental model following a trapezoidal rule [5].

The statistical analysis was performed using SAS System for Windows, version 9.1.2 (SAS Institute, Inc., Cary, NC, USA). Descriptive statistics was applied to the data of all randomized subjects who had received at least one dose of the investigational product. The data from test and reference products were tested by parametric and nonparametric approaches. Normality of distribution was tested with Shapiro-Wilk's test from the SAS UNIVARIATE procedure. A parametric (normal-theory) approach was applied for the analysis of log-transformed parameters (C_{max} , AUC_{0-t} and $AUC_{0-\infty}$). Non log-transformed parameters (T_{max} , λ_z and $T_{1/2}$) were evaluated by non-parametric tests. Schuirmann's two one-sided tests and the 90% confidence intervals were determined for the log-transformed parameters. The pharmacodynamic variables were treated similarly. Only those subjects who completed both study periods were included in the bioequivalence analysis. The formulations were considered bioequivalent when the difference between the compared primary pharmacokinetic (AUC_{0-24} , $AUC_{0-\infty}$ and C_{max}) and primary pharmacodynamic (ANC AUC_{0-72} , ANC $AUC_{0-\infty}$ and ANC $_{max}$) parameters was found statistically insignificant and the 90% confidence intervals were within the bioequivalence range of 80%–125% [6,7].

3. Results

3.1. Subjects' demographics and disposition

A total of 36 healthy male Caucasian subjects met all inclusion/exclusion criteria and were recruited. The age ranged from 18 to 41 years (23.0 ± 6.0 years, subjects' body weight was between 59 and 91 kg (76.6 ± 7.2 kg) and height was between 168 and 195 cm (178.5 ± 28.1 cm). One subject due to fulfillment to exclusion criteria before second study period (use of restricted medication) discontinued the study prematurely. Thirty-five volunteers completed the study and were considered for pharmacokinetic and pharmacodynamic evaluation.

3.2. Pharmacokinetics and pharmacodynamics

The mean plasma concentration-time profiles of test and reference filgrastim formulations after subcutaneous injection of 300 µg single dose is displayed in Fig. A. Baseline levels of G-CSF were not detected in any of the subjects. The

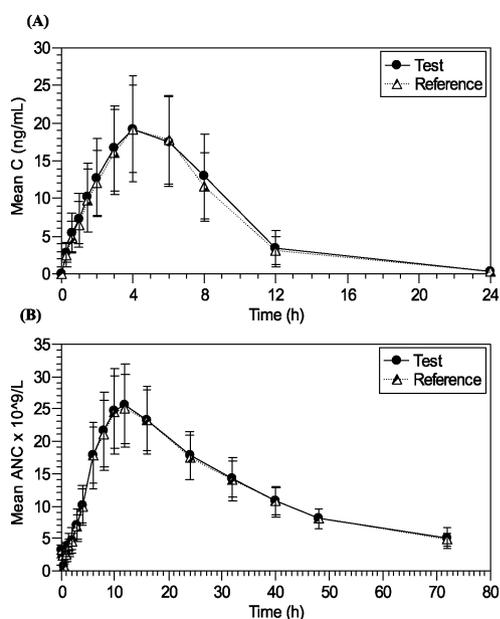


Fig. – Mean observed recombinant G-CSF concentration-time (A) and ANC-time profiles (B) after subcutaneous administration of 300 µg single dose of filgrastim to healthy volunteers.

concentration-time profiles of test and a reference formulations exhibited high similarity. The mean plasma G-CSF concentrations exhibited its peak after 4 h for both drug products (range 3.0–6.0 h) (Table 1). Thereafter, the concentrations decreased in monophasic manner in both dosage groups. Table 2 shows the results of statistical comparison of the primary pharmacokinetic parameters. The confidence intervals for the primary pharmacokinetic parameters were within 80%–125% interval. The nonparametric 90% confidence intervals for the pharmacokinetic parameters T_{max} , $T_{1/2}$ and λ_z were also enclosed in the $\pm 20\%$ acceptance range.

The mean ANC-time profiles of test and reference formulations are shown in Fig. B. The ANC-time profiles between the test and the reference formulations in both dosage groups were virtually superimposable. Initially, a fall in ANC below baseline was observed at 20 min to 1 h after administration of both test and reference formulations. Subsequently, ANC started to increase, passed baseline at around 90 min and further increased. The maximal ANC values were observed at around 12 h after administration. The increase in ANC for all subjects has returned to baseline values after 72 h (Fig. B). Table 2 shows the results of statistical comparison of the pharmacodynamic parameters in both treatment groups. The confidence intervals for the primary pharmacodynamic parameters were within 80%–125% interval and the nonparametric 90% confidence intervals for the pharmacodynamic parameter ANC t_{max} were within the $\pm 20\%$ acceptance range.

3.3. Safety

During the study, 24 subjects reported in total 41 adverse events (AEs): 14 subjects reported 21 AEs after administration of test formulation and 17 subjects reported 20 AEs after administration of reference formulation. The following AEs were reported most frequently: headache (13 events in 10 subjects), fever (9 events in 9 subjects) and back pain (3 events in 3 subjects). The most frequent AEs defined as laboratory abnormalities were: increase in ALT activity (3 events in 3 subjects) and hyperuricemia (2 events in 2 subjects).

Thirty-six AEs were reported as related to investigational products: 19 AEs after administration of the test and 17 AEs after the reference formulations. The AEs considered at least possibly related to the study drugs were (in the order of decreasing frequency): headache, fever, back pain, increased ALT, hyperuricemia, sweating, hypertension, increased phosphatemia, weakness.

The incidence of different AEs was similarly distributed across treatment groups.

Table 1 – Pharmacokinetic and pharmacodynamic parameters after subcutaneous administration of 300 µg of recombinant G-CSF to healthy volunteers.

| Parameter | Test formulation (SD) n = 35 | Reference formulation (SD) n = 35 |
|--|---------------------------------|--------------------------------------|
| AUC ₀₋₂₄ , ng × h/mL | 168.7 (56.6) | 161.7 (77.3) |
| AUC _{0-∞} , ng × h/mL | 170.5 (56.9) | 163.5 (47.5) |
| C _{max} , ng/mL | 20.3 (6.8) | 20.1 (5.7) |
| t _{max} , h | 4.6 (1.2) | 4.5 (1.0) |
| t _{1/2} , h | 3.3 (0.4) | 3.4 (0.5) |
| λ _z , h ⁻¹ | 0.2 (0.03) | 0.2 (0.03) |
| ANC AUC ₀₋₇₂ , h × 10 ⁹ /L | 894.7 (174.6) | 889.7 (150.6) |
| ANC AUC _{0-∞} , h × 10 ⁹ /L | 1086.83 (18.9) | 1074.7 (164.0) |
| ANC _{max} , 10 ⁹ /L | 25.9 (6.5) | 25.5 (5.9) |
| ANC t _{max} , h | 11.8 (1.7) | 11.8 (1.7) |

Values are mean (standard deviation).

C_{max}, peak concentration; AUC₀₋₂₄, the area under the concentration/time curve; AUC_{0-∞}, the area under the concentration/time curve extrapolated to infinity; t_{max}, time at which C_{max} occurred; t_{1/2}, the elimination half-life; λ_z, the elimination rate constant; ANC_{max}, the highest value of ANC determined; ANC AUC₀₋₇₂, the area under the blood ANC/time curve; ANC AUC_{0-∞}, the area under the blood ANC/time curve extrapolated to infinity; ANC t_{max}, time at which ANC_{max} occurred.

Table 2 – Statistical analysis of primary pharmacokinetic and pharmacodynamic parameters after subcutaneous administration of 300 µg of recombinant G-CSF to healthy volunteers.

| Parameter | T/R point estimate % (90% CI) | Intrasubject CV, % |
|--|-------------------------------|--------------------|
| AUC ₀₋₂₄ , ng × h/mL | 103.4 (95.6–111.9) | 19.73 |
| AUC _{0-∞} , ng × h/mL | 103.4 (95.7–111.7) | 19.20 |
| C _{max} , ng/mL | 99.6 (89.0–111.4) | 28.2 |
| ANC AUC ₀₋₇₂ , h × 10 ⁹ /L | 100.0 (96.6–103.5) | 8.57 |
| ANC AUC _{0-∞} , h × 10 ⁹ /L | 100.8 (96.5–105.3) | 10.73 |
| ANC _{max} , 10 ⁹ /L | 100.2 (95.4–105.1) | 11.95 |

C_{max}, peak concentration; AUC₀₋₂₄, the area under the concentration/time curve; AUC_{0-∞}, the area under the concentration/time curve extrapolated to infinity; ANC_{max}, the highest value of ANC determined; ANC AUC₀₋₇₂, the area under the blood ANC/time curve; ANC AUC_{0-∞}, the area under the blood ANC/time curve extrapolated to infinity.

4. Discussion

The standard scientific approach and regulatory rules for testing for bioequivalence were applied for this study in order to assess new biosimilar and reference filgrastim drugs in terms of bioavailability and pharmacological activity, by measuring G-CSF plasma concentration and ANC, which is a relevant pharmacodynamic biomarker of the efficacy of G-CSF drug products. Each formulation was administered as a single dose via the subcutaneous route in a cross-over design in two periods, separated by a wash-out phase of 14 days. The chosen design followed guidelines of the European Medicines Agency (EMA), published at the time of the study, including Guideline on the investigation of bioavailability and bioequivalence. There was no statistically significant carry-over effect for pharmacokinetic and pharmacodynamic variables, what can be considered as an indicator of adequate study design and conduct. To ensure a reliable estimate of the extent of drug absorption and of the effect on pharmacodynamic marker (ANC in this study), a blood sampling period of 3 half-lives was applied, as recommended by different guidelines. Mean extrapolated area was below 20% for test and reference formulations in both treatment groups (more than 80% of the AUC_{0-∞} and ANC AUC_{0-∞} was covered by the AUC₀₋₂₄ and ANC AUC₀₋₇₂ for test and reference formulations respectively). This indicates that the blood sampling collection period was adequate to characterize the pharmacokinetic and pharmacodynamic properties of the recombinant G-CSF.

In this study the 300 µg dose was chosen for a single-dose administration. It corresponded to the mean dose of 5 µg per kg of body weight. This is in compliance with the recommendation that the dose used in the bioequivalence study should be close to daily doses of the treatment of most therapeutic indications [8]. The selected doses were similar to the doses administered in other filgrastim studies in healthy volunteers [9–12]. Pharmacokinetic data obtained in this study are in line with those reported in previous studies, performed with filgrastim in healthy volunteers [12–14], as well as with the newly published data of new biosimilar filgrastim drugs [6,15].

The increases of ANC in study subjects in both dosage cohorts were preceded by initial decreases in neutrophils count over the first 20 min after administration of investigational products. The assumed mechanism responsible for this initial ANC decrease can be margination of the neutrophils to

endothelial cells [16]. The subsequent ANC increase following the initial decrease in ANC is considered to be caused by the release of mature neutrophils from the reserve pool of postmitotic mature myelocytes [17]. Both formulations effectively increased the ANC, the changes of this main pharmacodynamic marker of recombinant G-CSF were almost superimposable for 2 compared formulations. This could be explained by identical molecular structure and other physicochemical properties of two drugs, as demonstrated by comparability exercise and the same pharmaceutical formulation. ANC is considered as relevant biomarker for the efficacy of the product, as it is a diagnostic criterion of acute febrile neutropenia and also a predictor of the risk of infections in the patients with chemotherapy-induced neutropenia. Demonstration of pharmaceutical efficacy equivalence by ANC profile, together with pharmacokinetic equivalence and demonstration of the same well-defined physicochemical properties should be considered already to be a solid proof of the biosimilarity of filgrastim products.

The parameter ANC_{max} (25.9 × 10⁹/L) was similar in other clinical trials. Range of ANC_{max} was from 21.7 to 36.09 × 10⁹/L [10,11,19] [20]. ANC t_{max} (11.8 h) was not different in other clinical trials too. Range of ANC t_{max} was from 7.8 to 13.1 h [10,11,18,19]. Other study revealed ANC AUC_{0-∞} (1086.83 h × 10⁹/L), which was not different from our study established range – 945–1663 h × 10⁹/L [10,11,18,19].

The safety data obtained during this study indicate that single doses of 300 µg/mL of recombinant G-CSF were well tolerated. Main drug-related adverse events like headache, musculoskeletal pain, modification of laboratory tests are corresponding to those published for the originator product (SPC of Neupogen™) and of more recently developed other biosimilar filgrastim products [9–11,19].

5. Conclusions

The study results demonstrated the bioequivalence of two formulations Gp-02, a new biosimilar formulation of filgrastim, and the reference product Neupogen™ after administration of a 300 µg/mL dosage strength with respect to pharmacokinetic, pharmacodynamic and safety profiles. The observed pharmacokinetic and pharmacodynamic data support the biosimilarity of the new recombinant G-CSF to the reference medicinal product Neupogen™.

Conflict of interest

This study was financially supported by Sicor Biotech UAB (Teva Group). A.S., V.J., and R.K. contributed to the study design, the assessment and interpretation of data. G.G. was the principal investigator of the study. K.Š. was an employee of the Sicor Biotech (Teva Group) at the time of study. No other conflicts of interest are stated.

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