Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/medici

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

Audrius Sveikata [a,*], *Gintautas Gumbrevičius* [a], *Kastytis Šeštakauskas* [a], *Rima Kregždytė* [b], *Vytautas Janulionis* [c], *Vidmantas Fokas* [d]

[a] *Institute of Physiology and Pharmacology, Medicial Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania*
[b] *Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania*
[c] *Department of Applied Mathematics, Kaunas University of Technology, Kaunas, Lithuania*
[d] *"Biomapas" UAB, Kaunas, Lithuania*

ARTICLE INFO

Article history:
Received 9 November 2012
Accepted 23 June 2014
Available online 13 August 2014

Keywords:
Human granulocyte colony-stimulating factor
Biosimilarity
Pharmacokinetics
Pharmacodynamics
Safety

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 (Neupogen™) 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 STKS™ (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,

* *Corresponding author at:* Institute of Physiology and Pharmacology, Medical Academy, Lithuanian University of Health Sciences, A. Mickevičiaus 9, 44307 Kaunas, Lithuania.
E-mail address: audrius.sveikata@lsmuni.lt (A. Sveikata).

Peer review under responsibility of Lithuanian University of Health Sciences.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.medici.2014.08.001

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™.

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.