Biologicals 38 (2010) 557-566

Contents lists available at ScienceDirect

Biologicals



journal homepage: www.elsevier.com/locate/biologicals

Comparison of the physicochemical properties of a biosimilar filgrastim with those of reference filgrastim

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A R T I C L E I N F O

Article history: Received 13 January 2010 Received in revised form 23 April 2010 Accepted 26 May 2010

Keywords: Filgrastim Biosimilar G-CSF Neutropenia Physicochemical

ABSTRACT

Recombinant human granulocyte-colony stimulating factor (filgrastim) is a therapeutic protein used primarily to reduce incidence and duration of severe neutropenia and its associated, and serious, complications. We have developed a biosimilar filgrastim (Hospira filgrastim; NivestimTM) designed to be comparable to Amgen filgrastim (Neupogen[®]).

An extensive characterization study assessed the physiochemical similarity of Hospira filgrastim to Amgen filgrastim. Both drugs were supplied in 1 ml glass, single-use, prefilled syringes (five batches of each product at 480 μ g/0.5 ml and one batch of each product at 300 μ g/0.5 ml). Samples were evaluated using state-of-the-art analytical methods (validated in accordance with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use or Pharmeuropa guidelines) to determine physicochemical properties, molecular characteristics, purity and biological activity. Samples were compared after long-term storage at 2–8 °C and storage at 40 °C (stress conditions) to evaluate their degradation impurity profiles.

Hospira filgrastim and Amgen filgrastim were shown to have similar physicochemical properties, molecular characteristics, purity and biological activity. No significant differences in product-related impurities were recorded between Hospira filgrastim and Amgen filgrastim following storage for 12 weeks under stress conditions. These data show that the physicochemical profile of Hospira filgrastim is similar to that of Amgen filgrastim.

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1. Introduction

In the majority of cases, a single bioequivalence study is sufficient to demonstrate that a generic version of a small-molecule pharmaceutical is as safe and effective as its reference product [1], due, in part, to the relatively basic chemical structures of these molecules and the highly reproducible manufacturing processes. In contrast, biopharmaceuticals are extremely complex products, consisting of mixtures of highly similar molecules, with manufacturing processes that cannot be exactly replicated. As a result, 'generic' biopharmaceuticals do not exist, but legislation has enabled the development of similar biological medicinal products [2–4]. Biosimilars are considered to be mixtures of product variants that, although highly similar, may have an acceptable level of minor differences from the reference product.

Furthermore, there may be qualitative differences between the process-related impurities present in biosimilars and their reference products.

Over the next few years, the field of biosimilars is expected to rapidly expand with the imminent patent expiry of a number of important biopharmaceuticals. Filgrastim, a recombinant human granulocyte colony-stimulating factor (rhG-CSF), was first approved under the trade name Neupogen[®] (Amgen Inc., Thousand Oaks, CA, USA) in 1991, in both Europe and the US, and has already undergone patent expiry. Human G-CSF is a growth factor, produced by endothelial cells, macrophages and other immune cells, which influences the survival, proliferation and differentiation of cells of the neutrophil lineage [5]. In light of the haematopoietic activity of human G-CSF, filgrastim is primarily used to reduce the incidence and duration of neutropenia and associated complications.

Amgen filgrastim is a 175-amino acid recombinant protein with a molecular weight of 18 800 Da. While human G-CSF is a glycosylated protein, Amgen filgrastim is a non-glycosylated protein, produced in genetically modified *Escherichia coli* (*E. coli*). Its amino



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Table 1

Analytical methodologies employed for assessment of Hospira filgrastim and acceptance criteria for similarity with reference product (Amgen filgrastim).

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Ph. Eur. = European Pharmeuropa; IEF = isoelectric focusing; pl = protein isoelectric point; SDS-PAGE = sodium dodecyl sufate polyacrylamide gel electrophoresis; UV/VIS = ultraviolet-visible spectroscopy; RP-HPLC = reverse phase high-performance liquid chromatography; SEC-HPLC = size-exclusion high-performance liquid chromatography; IC = ion chromatography.

acid sequence is identical to that of human G-CSF, except for an additional N-terminal methionine.

A number of filgrastim biosimilars are currently approved for medicinal use or are in development. If comparable quality, safety and efficacy can be demonstrated to Amgen filgrastim, these agents could provide cost-effective alternatives. Here we report the data from a comparability study that was conducted to determine physicochemical and biological similarity between a biosimilar filgrastim (Hospira filgrastim; NivestimTM) manufactured and co-developed by PLIVA and Hospira, and reference filgrastim (Amgen filgrastim).

2. Materials and methods

Test product samples were: Hospira filgrastim 480 μ g/0.5 ml, lot numbers: 6241116, 6242116, 6243116 (24 months old at the time of testing), 4621067, 4626067 (17 months old at the time of testing); Hospira filgrastim 300 μ g/0.5 ml, lot: 4623107 (13 months old at the

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