Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
European Journal of Pharmaceutics and Biopharmaceutics 61 (2005) 142–148
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Pharmacokinetic comparison of two recombinant human granulocyte colony-stimulating factor after subcutaneous administration in rabbits

Jorge Ducongé[a,*], Leyanis Rodríguez-Vera[b], Carmen Valenzuela[c], Daniel Álvarez[a], Omar Ramírez[a], Kathya R. de la Luz-Hernández[d], Estela Y. Rabeza-Legón[d], Ángel Casacó[d], Eduardo Fernández-Sánchez[a]

[a]*Department of Pharmacology and Toxicology, Center for Biological Evaluation and Research, Institute of Pharmacy & Foods, University of Havana*
[b]*Department of Basic Chemistry, Institute of Pharmacy & Foods, University of Havana*
[c]*Clinical Trial Division, Center of Genetic Engineering and Biotechnology, Havana*
[d]*Center of Molecular Immunology, Havana*

Received 22 October 2004; accepted in revised form 20 June 2005
Available online 29 August 2005

Abstract

A pharmacokinetic comparison between two formulations (LeukoCIM, CIMAB SA versus Neupogen®, Hoffman-La Roche, licensed by Amgen) of recombinant human granulocyte colony-stimulating factor (rhG-CSF) using non-compartmental analysis was performed in male F1 rabbits after a single subcutaneous 11.5 μg/kg dose to help decide whether to conduct further comparability tests. Unlike the absorption phase, a statistical difference was not detected between Neupogen® and LeukoCIM for clearance (18.69 ± 11.83 versus 28.42 ± 12.11 mL/h/kg, $P = 0.22$). In addition, using a multivariate statistical analysis by independent samples test, a significant difference was not found between the two formulations ($P = 0.88$). Finally, the results obtained in this study confirmed the pharmacokinetic comparability between both formulations, supporting the claim for further assessments following the current protocol on biogeneric equivalence.

Keywords: Pharmacokinetics; Recombinant human granulocyte colony-stimulating factor; Clearance

1. Introduction

G-CSF was identified because it induced differentiation in a murine myelomonocytic leukemic cell line and stimulated granulocyte colony formation by normal progenitor cells [1]. The hematopoietic actions of this regulator are exclusive to cells of the neutrophilic granulocyte lineage [2,3]. Murine and human G-CSF have been purified from various sources [1–3] and the genes encoding human and murine G-CSF have been isolated [4–6]. Characterization of the gene encoding G-CSF has led to the production of the protein by recombinant DNA techniques. rhG-CSF is also capable of supporting the formation of granulocytic colonies from committed precursor cells. The broad species cross-reactivity of human G-CSF has allowed its in vivo action to be studied in mice [7], hamsters [8], rats [9], monkeys [10], and humans [11]. The predominant response in all species was a rapid dose-dependent neutrophilia. The availability of large quantities of molecularly homogeneous, biologically active human G-CSF by recombinant DNA technology has made it possible to explore the use of rhG-CSF as a therapeutic agent. Based on preclinical findings, rhG-CSF has been applied clinically to patients since the 1980s of last century in order to accelerate hematological recovery after high-dose chemotherapy with or without autologous bone marrow rescue, and it has been found to markedly shorten the duration of neutropenia [11–13]. Hence, rhG-CSF may be very useful in the treatment of cancer patients with drug- or irradiation-induced myelosuppression. Several reviews on G-CSF and its clinical applications have been published [14–16].

The potential marketplace for biological from different bioprocess modifications looms large. However, there is the widespread view that, for biopharmaceuticals, the process

* Corresponding author. School of Science and Technology, University of Turabo, PO Box 3030, Gurabo, PR 00778, USA. Tel.: +1 787 449 3692; fax: +1 787 728 2099.
E-mail address: jduconge@yahoo.com.mx (J. Ducongé).

0939-6411/$ - see front matter © 2005 Published by Elsevier B.V.
doi:10.1016/j.ejpb.2005.06.003

makes the product. The current guidelines include the physic-chemical and biological tests required to demonstrate structural equivalence of both products, the assessment of the potential impact of process changes on the biological quality of the products (using in vivo studies such as pharmacokinetic comparison), and the comparability in terms of safety and efficacy. Surprisingly, these guidelines were developed to facilitate changes in the processes applied by originators, but they have been extended to compare multisource biopharmaceuticals developed by different manufactures [17]. Although, we must recognize that, as written, the proposed draft leaves substantial room for interpretation. No universally applicable rules are being proposed, so each product must be reviewed on a case by case basis. As Enrico T Polastro and Arthur D Little argued, the regulatory authorities all over the world have become increasingly aware of the almost 'Kafkaesque' situation associated with this regulatory vacuum surrounding the comparability of complex molecules [18].

This paper describes the pharmacokinetic comparison in male F1 rabbits of two formulations of rhG-CSF [Filgrastim], developed and marketed by CIMAB SA, Cuba (LeukoCIM) and F Hoffman-La Roche, Switzerland, licensed by Amgen, USA (Neupogen®).

2. Materials and methods

2.1. Drug

LeukoCIM is a highly purified methionyl form of *E. coli*-expressed recombinant human G-CSF containing 175 amino acid residues that was developed by the Center of Molecular Immunology, Havana, Cuba, and is currently marketed by CIMAB SA. It has been produced following the standard of quality for injectable formulations, TRS 823 and 822 GMP regulations for pharmaceutical and biological drug products, respectively; and also following the Cuban norm 16/2000 from CECMED, Havana, Cuba (i.e. the cuban regulatory agency identified as The State Center for Drug Quality Control). Each vial contains 300 ($\pm$30) μg/mL (specific activity: 10^8 UI/mg proteins) of sterile recombinant human G-CSF.

Neupogen® brand name is a commercially available injectable formulation of human recombinant G-CSF [Filgrastim] developed and marketed by F Hoffman-La Roche, Switzerland, licensed by Amgen Inc. (Thousand Oaks, CA, USA). Each vial contains 300 ($\pm$30) μg/mL (specific activity: 10^8 UI/mg proteins) of filgrastim (Batch No. B1005).

2.2. Animal husbandry

Twelve male F1 rabbits (mean weight 2.1 kg; CENPALAB, Havana, Cuba) were housed individually in separate cages and also were bred and maintained under controlled conditions during the experiment (e.g. room temperature of 25 ± 2 °C and relative humidity of 65%). Access to food and water was provided ad libitum to all animals. All animal procedures were carried out under the approval of the Institutional Animal Care and Use Committee and were conducted in accordance with the standard operation procedures for good laboratory practices in animal use, at the Center for Biological Evaluations and Research, Havana, Cuba. All the invasive procedures were conducted under slight sodium pentobarbital anesthesia (40 mg/kg body weight). At the beginning of the experiment the animals were all randomly separated into two groups ($N=6$) for the pharmacokinetic characterization of either LeukoCIM or Neupogen® formulation, respectively.

2.3. Pharmacokinetic assays

Rabbits were administered subcutaneously at the dorsal region by a single dose of either LeukoCIM or Neupogen® product (11.5 μg/kg) following a parallel scheme. Blood samples (about 1.5 ml) were taken under slight anesthesia from the ear's marginal vein, at the following times: predose, 0.16, 0.25, 0.33, 0.42, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24, and 48 h postdose. All blood samples were collected in heparinized containers, and centrifuged at 4 °C for 10 min at $1000\times g$ within 30 min of collection. Then, they were separated into fractions and stored frozen at less than -70 °C until assay.

2.4. Human G-CSF assay

The plasma samples for human recombinant G-CSF quantification were measured using the commercially available Quantikine® G-CSF immunoassay DCS50 (R&D Systems, Inc., Minneapolis, MN, USA and distributed by R&D systems Europe, Abingdon, Oxon, UK) and a full description of this assay can be found elsewhere [19]. Briefly, this immunoassay is a 3.5–4.5 h solid phase sandwich-type Enzyme Linked ImmunoSorbent Assay (ELISA) technique designed to measure G-CSF in cell culture supernates, serum and plasma. It contains *E. coli*-expressed recombinant human G-CSF and horseradish peroxidase-conjugated polyclonal antibodies raised against the protein. It has been shown to accurately quantify recombinant human G-CSF in rabbit serum/plasma samples, with an intra-assay precision ranging from 1.1 to 2.8% CV and inter-assay precision ranging from 3.2 to 4.1% CV. The minimum detectable level (i.e. sensitivity) of G-CSF is typically <20 pg/mL. Results obtained showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards, indicating that the Quantikine Immunoassay kit can be used to determine relative mass values for human G-CSF. The assay has no measurable cross-reactivity or interference with other cytokines.

2.5. Pharmacokinetic analysis

The data analysis was performed by non-compartmental analysis (NCA) using a combined linear/log linear trapezoidal rule approach (WinNonlin professional software, Version 2.1, Pharsight Inc., 1997, NC, USA). A time zero value was considered for extrapolation purposes. The linear trapezoidal rule was used up to peak level, after which the logarithmic trapezoidal rule was applied. Lambda z is a first-order rate constant associated with the terminal (log linear) segment of the curve. It was estimated by linear regression of the terminal data points. The largest adjusted regression was selected in order to estimate lambda z, with one caveat: if the adjustment did not improve, but was rather within .0001 of the largest value, the regression with larger number of points was used.

For all data sets, model-independent metrics typically reported in pharmacokinetic studies were tabulated. Parameters extrapolated to infinity, using the moments of the curve, such as the area under the disposition curve, (AUC) the area under the first moment of the disposition curve, (AUMC) and mean residence time, (MRT) were computed based on the last predicted level, where the predicted value is based on the linear regression performed to estimate terminal lambda first-order rate constant. Computing these parameters based on the last observed level was discouraged in order to avoid larger estimation errors. Time to peak values were determined as the time of maximum observed level (i.e. maximum plasma concentration) considering the entire curve; and peak level is that corresponding to the above mentioned time to peak value. For all these purposes the WinNonlin professional software (Version 2.1, Pharsight Inc., 1997, NC, USA) was used.

Finally, the corresponding first-order absorption rate constants, denoted as Ka, were calculated following the classical Wagner–Nelson method, assuming first-order input and elimination, without lag-time. The algorithm was algebraically identical to that previously described by these two authors [20]. Briefly, the percentage remaining to be absorbed at each time point (PRA_T) was obtained through mass balance calculations, and then the semi-logarithmic plot of PRA_T versus time yielded an estimate of Ka.

2.6. Statistical analysis

All experimental data points were expressed as mean (± SD) from six animals per group. The corresponding average pharmacokinetic parameters (±SD) were calculated from individual estimates in each group. The assumptions of normality (Shapiro-Wilks test) and homogeneity of variances (Levene's test) were both verified. Comparison of average pharmacokinetic parameters from both groups were performed using either a parametric unpaired Student *t*-test for equality of means or a non-parametric U Mann Whitney test, depending on whether the assumption of normality can be proved. Finally, a multivariate analysis following the procedure earlier reported by Läuter and co-workers [21,22] was performed using the independent samples test, with a 95% confidence interval of the difference between both groups. Statistical significance was taken as P-value < 0.05. The statistical analyses were performed by SPSS for Windows, version 10.0 (SPSS Inc. Corp., 1999).

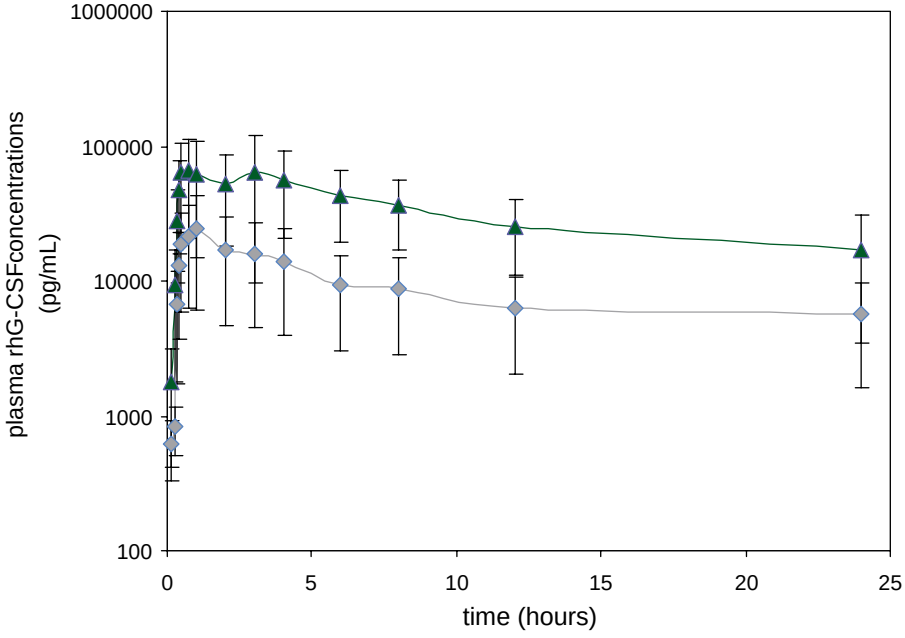

Fig. 1. Semilogarithmic plot of the observed mean (±SD) plasma rhG-CSF concentration over time after subcutaneous single dose input of 11.5 μg/kg rhG-CSF in F1 rabbits from both LeukoCIM formulation (full diamond, $N=6$) and Neupogen® formulation (full up-triangle, $N=5$).

Table 1
Pharmacokinetic parameters and metrics following non-compartmental analysis of the individual plasma LeukoCIM concentration data points after subcutaneous single dose input of 11.5 μg/kg rhG-CSF in F1 rabbits ($N=6$)

	$AUC_{0\rightarrow\infty}$ (ng·h/mL)	Peak level (ng/mL)	Time to peak (h)	CL/F (mL/h/kg)	Vss/F (mL)	λz half-life (h)	MRT (h)
1	311.5	33.21	4	36.92	640	5.3	8. 7
2	479.8	57.73	3	23.97	397	6.3	8.3
3	566.3	49.58	6	20.31	201	6.9	4.9
4	687.0	77.10	4	16.74	432	9.8	12.9
5	484.6	53.54	3	23.73	509	7.8	10.7
6	224.2	25.45	4	48.85	575	8.6	5.9
Mean	458.9	49.43	4	28.42	459	7.4	8.5
±SD	168.2	18.38	1.1	12.11	154.8	1.6	3.3
CV%	36.6	37.18	27.4	42.62	33.7	21.8	38.8

Linear-log linear trapezoidal rule was used.

3. Results

The pharmacokinetics of rhG-CSF after subcutaneous administration to male F1 rabbits was studied at a single dose of 11.5 μg protein/kg body weight. Fig. 1 depicts the mean (±SD) plasma recombinant human metionyl-GCSF [filgrastim] concentration time courses from both LeukoCIM and Neupogen® formulations. The individual pharmacokinetic parameters corresponding to each disposition profile from each formulation using non-compartmental analysis are presented in Tables 1 and 2, respectively. As can be seen, both formulations showed a rather similar kinetic pattern as judged by the shape of the curves depicting their systemic disposition, following a typical trajectory after subcutaneous input. However, it can be noted a slight up-and-down pattern being probably a consequence of the remarkable between-animal variability. Strikingly, this up-and-down pattern has been previously observed by Okabe and co-workers in normal male C3H/He mice by single intravenous injection of recombinant human granulocyte colony-stimulating factor [23]. The absorption rate was rather rapid after subcutaneous input of either LeukoCIM or Neupogen® formulations, even though time to peak was shorter when Neupogen® was given (1.8±1.5 versus 4±1.1 h). These two values lag behind those previously reported by Liu and Tang of 0.59±0.25 h after subcutaneous dose of iodinated rhGCSF in rabbits [24]. The observed plasma filgrastim concentration peaked to about 93±65 ng/mL for the Neupogen®, while the peak value was only 49±18 ng/mL for the LeukoCIM product, representing a difference of almost 2-fold between both formulations. Following the Wagner–Nelson method, first-order input kinetic without lag time assumed, the estimated absorption rate constant of $3.07\pm1.8\ h^{-1}$ for the Neupogen® was significantly faster ($P=0.03$) than that for the LeukoCIM ($0.74\pm0.5\ h^{-1}$), as can be seen in Table 3.

Interestingly, both systemic profiles showed a sharper decline after five hours, without any plateau being observed, and plasma withdrawn 48 h post-dose showed no detectable levels. The estimated elimination half-life parameters were no significant different each other ($P=0.142$) as reported in Tables 1 and 2, and they both were quite near the range reported by literature for filgrastim after subcutaneous and also intravenous dosing in rabbits (3–5 h) [24–26]. Likewise, clearance values (CL/F) in Tables 1 and 2 showed a rapid terminal disposition behavior at the elimination phase of this drug which suggested not significant difference ($P=0.22$) between both the Neupogen® brand name reference product in the marketplace and the LeukoCIM test product, developed by the Center of Molecular Immunology at Havana, Cuba. Notably, this elimination phase represents nearly 70% of the total area under drug

Table 2
Pharmacokinetic parameters and metrics following non-compartmental analysis of the individual plasma Neupogen® concentration data points after subcutaneous single dose input of 11.5 μg/kg rhG-CSF in F1 rabbits ($N=5$)

	$AUC_{0\rightarrow\infty}$ (ng·h/mL)	Peak level (ng/mL)	Time to peak (h)	CL/F (mL/h/kg)	Vss/F (mL)	λz half-life (h)	MRT (h)
1	436.7	29.11	0.5	26.33	305	4.6	5.8
2	1340.6	175.74	1	8.58	199	8.5	11.6
3	–	–	–	–	–	–	–
4	774.9	81.60	4	14.84	383	5.7	12.9
5	1375.1	144.52	3	8.36	159	5.6	9.5
6	325.3	33.96	0.75	35.35	318	2.9	4.5
Mean	850.5	92.98	1.8	18.69	272.8	5.4	8.8
±SD	491.9	65.57	1.5	11.83	91.7	2.0	3.6
CV%	57.8	70.51	84.1	63.28	33.6	37.5	40.7

Linear-log linear trapezoidal rule was used. Animal No. 3 was excluded because of diarrhea.

Table 3
Estimates of the first-order absorption rate constant by the classical Wagner–Nelson method using individual plasma drug concentration data sets from both LeukoCIM and Neupogen formulations, after subcutaneous single dose administration of 11.5 μg/kg rhG-CSF in F1 rabbits

	LeukoCIM Ka (h^{-1})	Neupogen® Ka (h^{-1})
1	1.52	4.96
2	0.77	1.56
3	0.25	–
4	0.87	0.91
5	0.35	3.19
6	0.71	4.74
Mean	0.74	3.07
±SD	0.45	1.82
CV%	60.6	59.4

Animal No. 3 belongs to the Neupogen®—treated group was excluded because of diarrhea.

concentration time curve, although absorption phase is accounting for most variability observed.

The estimated recombinant human metionyl-GCSF volume of distribution at steady-state were not significantly different ($P=0.081$) between the two formulations. Interestingly, there was no significant difference between the formulations as to the extent of systemic drug disposition ($P=0.20$), in spite of the fact that numerical differences were noticed by individual AUC metrics. It is probably due at least in part to their absorption differences, with remarkable variability in both cohorts. As was earlier mentioned, in contrast to this initial shorter absorption phase, no significant difference was detected between Neupogen® and LeukoCIM at the longer phase of elimination. The mean residence time parameters (MRT, $P=0.43$) showed in Tables 1 and 2 are supporting this statement.

Finally, considering the high correlation among pharmacokinetic parameters, a multivariate statistical analysis of the estimated parameters using the standardized sums test was performed, and once again no significant difference was found between these two formulations ($P=0.88$). As a result, we were encouraged to stress the idea of 'sameness' between both formulations of recombinant human metionyl-GCSF under study (i.e. LeukoCIM, CIMAB SA, Cuba, and Neupogen®, Hoffman-La Roche, Switzerland, licensed by Amgen®, USA).

4. Discussion

The pharmacokinetics of rhG-CSF in animals and humans has been reported using various assay techniques for rhG-CSF, or iodinated rhG-CSF, determination in plasma and serum [8,9,12,24,27]. Although a good agreement between different assays (e.g. [^{3}H] thymidine uptake assay and the sandwich enzyme-linked immunosorbent assay) have been observed with blood samples after administration of rhG-CSF, some authors have recognized the impact of such an issue on reported pharmacokinetic difference among experiments at different labs [28]. Indeed, in their comments on paper by Okabe et al. [23], Tanaka and Kaneko [28] argued that the observed difference between both studies appear to be rather a consequence of the selected assay than an actual pharmacokinetic disparity. In this sense, some observed discrepancies between earlier reported values in literature by others and the results here discussed could probably be a matter of methods.

The results provide evidence in order to support the idea of a rapid systemic clearance of filgrastim from blood compartment, probably due to the combination of a G-CSF receptor-mediated endocytosis (RME) saturable clearance process at bone marrow and plasma protease cleavage with subsequent glomerular filtration [23,29,30], without any relevant drug distribution from blood into the innermost tissues. As a matter of fact, the estimated Vss/F values will be larger than real Vss parameter if we take into account the bioavailability metric ($F\leq 1$) after subcutaneous administration of filgrastim from both formulations. In fact, relatively small values of volume of distribution have been previously observed in other mammalian species such as rats [9,27].

It has been suggested that the effects of G-CSF on hematopoiesis are more positively influenced by the duration of G-CSF presence in plasma than by the peak G-CSF concentration. Accordingly, the results here discussed are in a good agreement with our statement of pharmacokinetic comparability between both preparations, considering the relevance of the estimated parameters other than absorption ones. Actually, significant differences between the products were only observed during the initial absorption phase, whereas their rapid elimination rates were quite similar each other. So, those pharmacokinetic parameters for elimination rate and drug duration time in blood (e.g. elimination half-life, MRT, systemic clearance) should be highlighted for any decision-making.

In general, most authors assume that small proteins (viz., G-CSF is about 20 KDa glycoprotein hormone) are eliminated from circulatory blood only by glomerular filtration and proteolysis [31,32]. Recently, it has been postulated that an important factor governing the pharmacokinetics of cytokines and growth factors such as G-CSF is the key role played by the cell-surface receptor in their overall elimination from the body [30,33]. For these cytokines, the clearance organ is at the same time the target organ (viz., bone marrow for the G-CSF). Thus, RME is now recognized as one of their primary clearance mechanisms.

In fact, Kuwabara et al. [34] revealed that rhG-CSF is eliminated through two pathways in humans, monkeys, rats and probably other species: a saturable tissue uptake clearance process, which is mediated by the G-CSF receptor, located mainly in the bone marrow, and an unsaturable clearance process. In this regard, the clearance of rhG-CSF is known to decrease with increasing dose [27,34,35], which could explain the faster clearance

observed in this study in relation to previous reports in the literature using higher doses (50–100 μg protein/ kg body weight).

Interestingly, this saturable process has been demonstrated to have a close relationship to the amount consumed by receptors in the bone marrow per time unit (e.g. estimated as about half of the dose by Hayashi et al. [29], as suggested by the positive correlation between the elimination rate constant and the neutrophil increase). Consequently, despite the difference observed for absorption, it seems to be a logical assumption that if the clearance of G-CSF by receptor-mediated endocytosis (i.e. 80% of total systemic G-CSF clearance, according to literature) is no statistically different, then the rate of occupancy of G-CSF receptors could be expected to be no significant different between both groups. In doing so, it is important to keep in mind that binding is a previous requisite to internalization and degradation of the ligand-receptor complex (i.e. a rate-limiting step; 770 min versus 30 and 20 min half-times, respectively) and the rate constants for the RME processes determined in different experiments are similar [36]. Therefore, we are encouraged to postulate a potential similarity in efficacy between both preparations as neutrophil counts increment is a biological outcome of the G-CSF action upon binding to G-CSF receptor (i.e. occupancy).

Historically, biologics have been regulated a world apart from classically synthesized drugs. Limited analytical tools and product-specific processes galvanized the long-standing approach that biologics were defined by the manufacturing process. Thus, the 'product = process' dogma has permeated throughout biologics development. It was this process-specific distinction more than anything else what blurred the remarkable future of some biotech-derived drug products obtained by non-conventional technology (i.e. bioprocess modifications), even if the active ingredient is identical to that derived by 'conventional technology'. The premise of this policy is that biotechnology has the potential to induce new structural features in the product, lead to microheterogeneity (e.g. glycosylation pattern), or introduce new contaminants, each of which could impact the safety, efficacy, or stability of the product.

Indeed, such changes, at least as seen by regulators who are vested with the task of overseeing the safety of pharmaceuticals, could potentially result in an altered safety and efficacy profile. This would require extensive, and also expensive, clinical trials to gain regulatory approval. However, this situation is evolving. The dogma that the 'process makes the product' has been increasingly challenged. It appears that-if not strictly identical- at least comparable biologics can be obtained through different processes. This is the case for most well-characterized proteins. An interesting example is the case of the Human Growth Hormone (HGH), which is being produced in Europe in a variety of expression systems (i.e. standard *E. coli*, special strains of *E. coli* and transformed mouse cell lines), with identical results, a 191-amino acid sequence copying the human pituitary growth hormone. Thus, there are at least five HGH products currently on the European market, each obtained by different companies (Pharmacia Ferring, Lilly Novo, Serono, etc.) based on distinct processes and even involving different expression systems. All of these products appear to show the same profiles in terms of amino acid sequence, potency and safety and efficacy, undermining the belief that 'the process makes the product' [18].

The point that any change in process, methods, or specifications would preclude any comparability seems especially ironic since innovator manufacturers have successfully supported an opposite rationale for their continual process improvements since 1982 [37]. In this context, can there be product differences arising from the two processes that cannot be detected by current analytical methods or pharmacokinetic evaluations but still have an impact on safety and efficacy? The answer is sometimes an obvious double standard.

Lastly, that is exactly what we want to stress here in order to support our concluding remark concerning the impact of the observed pre-clinical pharmacokinetic similarity between these two rhG-CSF formulations under study on decision-making in relation to their further clinical comparability taking into consideration the broad species cross-reactivity of human G-CSF.

Acknowledgements

This work was supported by the Center of Molecular Immunology, Havana, Cuba. The authors would like to thank the technical assistance of Ms Maria Bécquer, Mr Miguel de Dios Oliva, Mr. Carlos Muñoz, and Miss. Wilnelia López for their excellent contributions to this investigation.

References

[1] N.A. Nicola, D. Metclaf, M. Matsumoto, G.R. Johnson, Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells. Identification as granulocyte colony-stimulating factor, J. Biol. Chem. 258 (1983) 9017–9023.

[2] D. Metcalf, N.A. Nicola, Proliferative effects of purified granulocyte colony-stimulating factor (G-CSF) on normal mouse hemopoietic cells, J. Cell Physiol. 116 (1983) 198–206.

[3] K. Welte, E. Platzer, L. Lu, J. Gabrilove, E. Levi, R. Mertelsmann, M.A.S. Moore, Purification and biochemical characterization of human pluripotent hematopoietic colony-stimulating factor, Proc. Natl. Acad. Sci. USA 82 (1985) 1526–1530.

[4] L.M. Souza, T.C. Boone, J. Gabrilove, P.H. Lai, K.M. Zsebo, D.C. Murdock, V.R. Chazin, J. Bruszewski, H. Lu, K.K. Chen, J. Barendt, E. Platzer, M.A.S. Moore, R. Mertelsmann, K. Welte, Recombinant human G-CSF: effects on normal and leukemic myeloid cells, Science (Washington DC) 232 (1986) 61–65.

[5] S. Nagata, M. Tsuchiya, S. Asano, Y. Kaziro, T. Yamazaki, O. Yamamoto, Y. Hirata, N. Kubota, M. Oheda, H. Nombra, M. Ono, Molecular cloning and expression of cDNA for human G-CSF, Nature (Lond.) 319 (1986) 415–418.
[6] M. Tsuchiya, S. Asano, Y. Kaziro, S. Nagata, Isolation and characterization of the cDNA for murine G-CSF, Proc. Natl. Acad. Sci. USA 83 (1986) 7633–7637.
[7] Z. Podja, G. Molineux, T.M. Dexter, Hemopoietic effects of short-term in vivo treatment of mice with various doses of rhG-CSF, Exp. Hematol. 18 (1990) 27–31.
[8] A.M. Cohen, K.M. Zsebo, H. Inoue, D. Hines, T.C. Boone, V.R. Chazin, L. Tsai, T. Ritch, L.M. Souza, In vivo stimulation of granulopoiesis by recombinant human G-CSF, Proc. Nat. Acad. Sci. USA 84 (1987) 2484–2488.
[9] H. Tanaka, Y. Okada, M. Kawagishi, T. Tokiwa, Pharmacokinetics and pharmacodynamics of recombinant human G-CSF after intravenous and subcutaneous administration in the rat, J. Pharmacol. Exp. Ther. 251 (1989) 1199–1203.
[10] K. Welte, A.M. Bonilla, A.P. Gillio, T.C. Boone, G.K. Potter, J.L. Gabrilove, M.A.S. Moore, R.J. O'Reilly, L.M. Souza, Recombinant human G-CSF. Effects on hematopoiesis in normal and cyclophosphamide-treated primates, J. Exp. Med. 165 (1987) 941–948.
[11] M.H. Bronchud, J.H. Scarffe, N. Thatcher, D. Crowther, L.M. Souza, N.K. Alton, N.G. Testa, T.M. Dexter, Phase I/II study of recombinant human G-CSF in patients receiving intensive chemotherapy for small cell lung cancer, Br. J. Cancer 56 (1987) 809–813.
[12] G. Morstyn, L. Campbell, L.M. Souza, N.K. Alton, J. Keech, M. Green, W. Sheridan, D. Metclaf, R. Fox, Effect of G-CSF on neutropenia indiced by cytotoxic chemotherapy, Lancet 1 (1988) 667–672.
[13] H. Teshima, J. Isikawa, H. Kitayama, T. Yamagami, A. Hiraoka, H. Nakamura, H. Shibata, T. Masaoka, F. Takaku, Clinical effects of rhG-CSF in leukemia patients: a phase I/II study, Exp. Hematol. 17 (1989) 853–858.
[14] S. Nagata, in: M.B. Sporn, A.B. Roberts (Eds.), Peptide growth factors and their receptors, Springer, New York, USA, 1990, p. 190.
[15] S. Nagata, in: A. Thompson (Ed.), The cytokine handbook2nd ed, Academic Press, New York, USA, 1994, p. 371.
[16] J.L. Gabrilove, Clinical applications of granulocyte colony stimulating factor (G-CSF), Growth Factors 6 (1992) 187–191.
[17] FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, including Therapeutic Biotechnology-derived Products, July 1996, CBER, Rockville, MD, USA, http://www.fda.gov/cder/compare.htm, revised April 2003.
[18] E.T. Polastro, A.D. Little, The future of biogenerics: When will we see legal generics of top biopharmaceuticals?, http://www.contractpharma.com/oct013.htm, revised November 2003.
[19] Catalog DCS50, Quantikine Human G-CSF Immunoassay, For the quantitative determination of human granulocyte colony stimulating factor concentrations in cell culture supernate, serum and plasma, available at http://www.rndsystems.com, revised April 2003.
[20] J.G. Wagner, E. Nelson, Kinetic analysis of blood levels and urinary excretion in the absorptive phase after single doses of drugs, J. Pharm. Sci. 53 (1964) 1392–1403.
[21] J. Läuter, Exact t- and F-tests for analyzing studies with multiple endpoints, Biometrics 52 (1996) 964–970.
[22] J. Läuter, E. Glimm, S. Kropf, New multivariate test for data with an inherent structure, Biometrical J 38 (1996) 5–23.
[23] M. Okabe, M. Asano, T. Kuga, Y. Komatsu, M. Yamasaki, Y. Yokoo, S. Itoh, M. Morimoto, T. Oka, In vitro and in vivo hematopoietic effect of mutant human granulocyte colony stimulating factor, Blood 75 (9) (1990) 1788–1793.
[24] X.W. Liu, Z.M. Tang, Pharmacokinetic of recombinant human granulocyte colony stimulating factor in rabbits and mice, Zhongguo Yao Li Xue Bao 18 (1) (1997) 44–48.
[25] Product Information. F Hoffman-La Roche SA, Basilea, Switzerland, 1998.
[26] Neupogen® product, Amgen® Inc. Thousand Oaks, California, 91320-1789, 1994.
[27] H. Tanaka, T. Tokiwa, Pharmacokinetics of recombinant human G-CSF studied in the rat by a sandwich enzyme-linked immunosorbent assay, J. Pharmacol. Exp. Ther. 255 (1990) 724–729.
[28] H. Tanaka, T. Kaneko, Pharmacokinetics of recombinant human G-CSF in mice, Blood 79 (2) (1992) 536–539.
[29] N. Hayashi, H. Aso, M. Higashida, H. Kinoshita, S. Ohdo, E. Yukawa, S. Higuchi, Estimation of rhG-CSF absorption kinetics after subcutaneous administration using a modified Wagner-Nelson method with a nonlinear elimination model, Eur. J. Pharm. Sci. 13 (2001) 151–158.
[30] Y. Kato, T. Seita, T. Kuwabara, Y. Sugiyama, Kinetic analysis of receptor-mediated endocytosis (RME) of proteins and peptides: Use of RME as a drug delivery system, J. Controlled Release 39 (1996) 191–200.
[31] W. Strober, T.A. Waldmann, The role of the kidney in the metabolism of plasma proteins, Nephron 13 (1974) 35–66.
[32] T. Maack, V. Johnson, S.T. Kau, J. Figneiredo, D. Sigulem, Renal filtration, transport and metabolism of low-molecular weight proteins: a review, Kidney Int. 16 (1979) 251–270.
[33] T. Kuwabara, T. Uchimura, H. Kobayashi, S. Kobayashi, Y. Sugiyama, Receptor-mediated clearance of G-CSF derivative nartograstim in bone marrow, Am. J. Physiol. (Endocrinol. Metab.) 269 (1995) E1–E9.
[34] T. Kuwabara, Y. Kato, S. Kobayashi, H. Suzuki, Y. Sugiyama, Nonlinear pharmacokinetics of a colony-stimulating factor derivative (nartograstim). Species differences among rats, monkeys and humans, J. Pharmacol. Exp. Ther. 271 (1994) 1535–1543.
[35] A.C. Houston, L.A. Stevens, V. Cour, Pharmacokinetics of glycosylated recombinant human G-CSF (lenograstim) in healthy male volunteers, Br. J. Clin. Pharmacol. 47 (1999) 279–284.
[36] T. Kuwabara, S. Kobayashi, Y. Sugiyama, Kinetic analysis of receptor-mediated endocytosis of G-CSF derivative, nartograstim, in rat bone marrow cells, Am. J. Physiol. 271 (1996) E73–E84.
[37] R.L. Zeid, Regulatory and development issues in the demonstration of therapeutic equivalence for multisource biotech-derived pharmaceuticals, Drug Information J 34 (2000) 919–959.