A pharmacokinetic model of filgrastim and pegfilgrastim application in normal mice and those with cyclophosphamide-induced granulocytopenia

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Abstract

Objectives: Recombinant human granulocyte colony-stimulating factor (rhG-CSF) is widely used as treatment for granulocytopenia during cytotoxic chemotherapy; however, optimal scheduling of this pharmaceutical is unknown. Biomathematical models can help to pre-select optimal application schedules but precise pharmacokinetic properties of the pharmaceuticals are required at first. In this study, we have aimed to construct a pharmacokinetic model of G-CSF derivatives filgrastim and pegfilgrastim in mice.

Methods: Healthy CD-1 mice and those with cyclophosphamide-induced granulocytopenia were studied after administration of filgrastim and pegfilgrastim in different dosing and timing schedules. Close meshed time series of granulocytes and G-CSF plasma concentrations were determined. An ordinary differential equations model of pharmacokinetics was constructed on the basis of known mechanisms of drug distribution and degradation.

Results: Predictions of the model fit well with all experimental data for both filgrastim and pegfilgrastim. We obtained a unique parameter setting for all experimental scenarios. Differences in pharmacokinetics between filgrastim and pegfilgrastim can be explained by different estimates of model parameters rather than by different model mechanisms. Parameter estimates with respect to distribution and clear-

ance of the drug derivatives are in agreement with qualitative experimental results.

Conclusion: Dynamics of filgrastim and pegfilgrastim plasma levels can be explained by the same pharmacokinetic model but different model parameters. Because of a strong clearance mechanism mediated by granulocytes, granulocytotic and granulocytopenic conditions must be studied simultaneously to construct a reliable model. The pharmacokinetic model will be extended to a murine model of granulopoiesis under chemotherapy and G-CSF application.

Introduction

Cytotoxic chemotherapies can lead to several cytopenic side effects. Severe neutropenia is a specially serious clinical issue because it is associated with increased incidence of infections, hospitalization, antibiotic treatment, reduction of therapy intensity, therapy dropouts and therapy-associated deaths (1–6). Recombinant human granulocyte colony-stimulating factor (rhG-CSF) is routinely applied to ameliorate or prevent neutropenia (1–7); it acts by mitotic activation of granulopoietic progenitors and precursors, accelerating maturation of cells and improving release of mature granulocytes from the bone marrow (8–14).

Filgrastim was the first pharmaceutical rhG-CSF; it has a short half-life *in vivo* because of strong renal elimination and specific degradation mediated by G-CSF receptors or neutrophil elastase (15–22). Therefore, filgrastim must be applied multiple times during one cycle of chemotherapy. Pegylated filgrastim (pegfilgrastim) is the next generation of rhG-CSF; it shows prolonged half-life *in vivo* due to reduced renal clearance (23–25). In clinical practice, it is believed that a single injection of pegfilgrastim is at least as effective as multiple injections of filgrastim with respect to treating neutropenia and preventing infection (2,26–29).

However, the effectiveness of G-CSF treatment depends on many variable therapy parameters, such as

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applied chemotherapy, individual factors and, especially, dosing and timing of G-CSF itself (30). Because of this large set of variable therapy parameters, optimal G-CSF treatment is not obvious and cannot be developed only on the basis of clinical trials.

Previously, we have shown that a biomathematical model of human granulopoiesis, including a preliminary pharmacokinetic model of filgrastim application, is a valuable tool to pre-select optimal filgrastim schedules in various therapy situations (31–33). We aim to include the new drug pegfilgrastim in our model in order to optimize corresponding dosing and timing schedules. However, differences in pharmacokinetics (PK) and pharmacodynamics (PD) between filgrastim and pegfilgrastim have not been analysed well enough.

An important PK characteristic of both derivatives is the strong interaction between G-CSF-induced granulocytosis and specific degradation of G-CSF mediated by granulocytes themselves. Therefore, close meshed time series data of both G-CSF plasma concentrations and granulocytes under various therapy conditions are required to construct a PK model of filgrastim and pegfilgrastim.

On the basis of the time series data of experiments specifically performed for modelling purposes, we have constructed such a model in the murine situation first. Experiments comprise measuring G-CSF plasma concentrations under various G-CSF schedules and with or without chemotherapy. We used the same PK model structure for both filgrastim and pegfilgrastim. Hence, differences between the drugs can be reduced to differences in model parameters, which allow comparisons of the PK properties between the two drugs.

The resulting model is the first one that explains PK properties of filgrastim and pegfilgrastim under granulocytotic and granulocytopenic conditions in the framework of one comprehensive model. The model can be used to predict G-CSF plasma concentration given the application schedule of the drug and the resulting granulocyte dynamics. Later, this model will be extended to a fully data-independent PK and PD model of granulopoiesis under chemotherapy and G-CSF application.

Materials and Methods

Experimental interventions

We used 8-week-old, female, outbred Hsd:ICR mice (CD-1[®], Harlan Sprague Dawley Inc., Indianapolis, IN, USA, obtained from a breeding stock from Charles River Breeding Laboratories Wilmington, MA, USA) for this study; they were maintained in a defined flora animal facility.

Multiple doses of filgrastim (10 or 20 µg; Neupogen) or single doses of pegfilgrastim (40 µg; Neulasta, both

from Amgen, Thousand Oaks, CA, USA) were injected subcutaneously between the scapulae. All mass data regarding rhG-CSF applications are given as protein mass. Cyclophosphamide (450 mg/kg body weight, 12 mg per animal; Endoxan, Baxter Deutschland GmbH, Unterschleissheim, Germany) was injected intraperitoneally at an injection volume of 200 µl.

Peripheral blood of the mice was taken from the tail vein at specified time points. Blood was collected through heparinized capillaries (Rolf Greiner Biochemica GmbH, Fracht, Germany) and transferred to heparinized tubes (Heparin-Natrium-25 000, Ratiopharm GmbH, Ulm, Germany). Counts of circulating granulocytes were determined using an animal blood counter (scil Vet abc, SCIL animal care company GmbH, Viernheim, Germany), which had been calibrated for mouse blood.

G-CSF plasma levels were measured using the human G-CSF Cytometric Bead Array kit (CBA, BD Biosciences, San Diego, CA, USA). Data were acquired on FACS Calibur instrumentation (Becton Dickinson, Franklin Lakes, NJ, USA) and was analysed using FCAP Array software (BD Biosciences).

All measurements were taken from at least five mice.

Study design

In order to construct the PK model of G-CSF, including high dependence of degradation on circulating granulocyte dynamics, it is necessary to study the time series of drug plasma concentrations of different G-CSF schedules under granulocytopenic and granulocytotic conditions.

Thus, we applied three doses of 10 and 20 µg of filgrastim on consecutive days to healthy mice, in order to study PK of G-CSF under elevated granulocyte counts. Alternatively, we applied a fixed single dose of either 20 or 40 µg pegfilgrastim into healthy mice. For experiments with pegfilgrastim, we used two groups of animals comprising five individuals each. For the experiments with filgrastim, we used four groups of animals. Taking of blood samples was time-shifted between the groups, resulting in a total of two or four measurements per day, respectively.

Additionally, we performed experiments with combined application of cyclophosphamide and G-CSF. After a fixed dose of cyclophosphamide (12 mg), we applied two doses of 20 µg filgrastim (2×20 µg) or four doses of 10 µg filgrastim (4×10 µg) on consecutive days after cyclophosphamide application. Treatment was started directly (1 h later), 1 or 2 days after cyclophosphamide. Alternatively, we applied a fixed single dose of 40 µg pegfilgrastim directly (1 h later), 1 or 2 days after cyclophosphamide.

Modelling work

We constructed a PK model of both filgrastim and pegfilgrastim. For this purpose, physiological knowledge about absorption, distribution and elimination kinetics of the G-CSF derivatives has been transformed into ordinary differential equations. Basic structure of the model is based on a preliminary PK model of filgrastim application in humans (32).

We used the same model equations but different parameters for each drug derivative. Unknown parameters were determined by fitting the model to measured time courses of G-CSF plasma concentrations, using evolutionary strategies (34). We have aimed to minimize the absolute difference between model and geometric mean of the data. In order to assess identifiability of parameters and precision of parameter estimates, we calculated a kind of univariate confidence interval for all parameter estimates. This interval is determined by the range of values that would result in a fitness deterioration of at most 10% compared to optimal fitness.

Since elimination of drugs is mainly determined by numbers of circulating granulocytes (for example, 15,17–19,35–37, among others), measured granulocyte counts have been linearly interpolated and imprinted into the PK model. This approach lead to a model that predicts G-CSF plasma concentrations when G-CSF scheduling and granulocyte time course is known.

Differential equations and simulations have been implemented in the mathematical software package MATLAB 7.5.0.342 with SIMULINK toolbox (The MathWorks Inc., Natick, MA, USA).

Results

Experimental data

Time series data for healthy mice can be found in Fig. 1. Application of both derivatives resulted in strong granulocytosis, which quickly diminished 24 h after the last filgrastim application but continued with pegfilgrastim until day 5, where cell counts had been normalized. Over-compensation of granulocytosis could be recognized for filgrastim but not for pegfilgrastim. Filgrastim plasma concentration fluctuated quickly between applications, while pegfilgrastim plasma concentration remained high for a plateau phase of approximately 2 days then dropped quickly afterwards.

Data of G-CSF plasma concentration and granulocyte counts in mice with cyclophosphamide-induced granulocytopenia are presented in Fig. 2. Depth and duration of the granulocytopenic phase depended heavily on G-CSF scheduling used. Schedules $4 \times 10 \mu\text{g}$ filgrastim at day 1 or $40 \mu\text{g}$ pegfilgrastim at day 1 showed lowest granulotoxicity. Over-compensation of granulocytopenia was found for all pegfilgrastim schedules and for filgrastim day 2, but not

for the other filgrastim schedules. Detailed analysis of haematotoxicity of all schedules can be found elsewhere (38).

PK model

The following compartments were considered in the model: subcutaneous tissue in which drugs were injected, the central compartment representing blood plasma in which drugs are detectable, and a peripheral compartment where drugs are temporarily removed from the central compartment (for example, due to protein binding) (39). Dynamics of G-CSF plasma concentrations can be modelled by fluxes between these compartments and degradation processes also. The basic structure of the model can be found in Fig. 3.

Major features of the model are delayed influx from subcutaneous tissue to the central compartment, which is modelled by a gamma-distributed absorption kinetics, represented by a fixed number of delay compartments (see Scholz *et al.* for further details (32)), non-specific elimination from the central compartment (renal clearance) modelled by a first-order elimination, specific elimination from the central compartment which is proportional to both, absolute granulocyte count and a Michaelis-Menten term as well and exchange between central and peripheral compartments modelled by a first-order transition (reversible protein binding). More specifically, these properties have been transformed into an ordinary differential equation system describing the change of compartment contents over time. The system has the following form, starting with the subcutaneous tissue:

$$\frac{dC_{G-CSF}^{sc-1}}{dt} = \text{Rate} - k_{sc} C_{G-CSF}^{sc-1} \quad (1)$$

$$\frac{dC_{G-CSF}^{sc-i}}{dt} = k_{sc} C_{G-CSF}^{sc-(i-1)} - k_{sc} C_{G-CSF}^{sc-i}, \quad 2 \leq i \leq N \quad (2)$$

The subcutaneous compartment is divided into N identical sub-compartments to model lag of influx of drugs into the central compartment, resulting in a gamma-distributed lag time with expectation N/k_{sc} and variance N/k_{sc}^2 (compare Scholz *et al.* (32)). We chose $N = 5$ but changing k_{sc} adequately; a number of sub-compartments between 2 and 10 would also result in good model fits (not shown). G-CSF is injected in the first sub-compartment, which can be modelled by an injection function Rate , which is positive and constant during injection time t_{inj} and zero elsewhere.

The equation for the central compartment has the form:

$$\begin{aligned} \frac{dC_{G-CSF}^{cent}}{dt} = & k_{sc} C_{G-CSF}^{sc-N} + k_{21} C_{G-CSF}^{per} - k_{12} C_{G-CSF}^{cent} \\ & - k_{el}^{cent} C_{G-CSF}^{cent} - k_s^{GRA} \text{GRA} \frac{C_{G-CSF}^{cent}}{C_{G-CSF}^{cent} + k_m^{GRA}}, \end{aligned} \quad (3)$$

where the first summand on the right-hand side is influx from the subcutaneous compartment. The second and

Figure 1. Application of granulocyte colony-stimulating factor (G-CSF, arrows) into healthy mice. Data are presented as geometric mean and standard deviation, dotted line for granulocyte counts marks the normal value. Note that time scales are different between the figures: (a) granulocyte dynamics after application of $3 \times 10 \mu\text{g}$ and $3 \times 20 \mu\text{g}$ filgrastim on consecutive days; (b) comparison of corresponding plasma concentrations of filgrastim (data points) with predictions of the pharmacokinetic model (lines); (c) granulocyte dynamics after application of a single dose of 20 or $40 \mu\text{g}$ of pegfilgrastim; and (d) comparison of plasma concentrations of pegfilgrastim (data points) with predictions of the pharmacokinetic model (lines).

the third summands describe exchange of drugs between central and peripheral compartments modelled by a first-order transition (C_{G-CSF}^{per} influx from peripheral to central, C_{G-CSF}^{cent} efflux from central to peripheral compartment). The fourth summand is the model for first-order non-specific (renal) elimination of the drugs and the fifth term is specific degradation mediated by granulocyte count GRA . This mechanism is modelled by Michaelis-Menten elimination kinetics. Content of the central compartment C_{G-CSF}^{cent} is divided by distribution volume (vol) in order to compare model predictions with measured G-CSF plasma concentrations.

The equation for the peripheral compartment has the simple form:

$$\frac{dC_{G-CSF}^{per}}{dt} = k_{12}C_{G-CSF}^{cent} - k_{21}C_{G-CSF}^{per}, \quad (4)$$

which only describes a first-order transition between central and peripheral compartments.

Initial values of all equations are zero.

Model fitting resulted in an excellent fit of all scenarios with granulocytopenic mice and also the data set of healthy mice (Figs 1 and 2).

An overview of all model parameters and quantities can be found in Table 1. All fitted parameters showed good identifiability indicated by narrow confidence intervals. Hence, there are no redundant parameters in the model and precision of all parameters is high.

In comparison to filgrastim, we estimated that pegfilgrastim has an approximately double transition time through subcutaneous tissue. Furthermore, distribution volume and the non-specific elimination are lower for pegfilgrastim;

Figure 2. Application of granulocyte colony-stimulating factor (G-CSF) into mice with cyclophosphamide-induced granulocytopenia (12 mg cyclophosphamide at day 0). Data are presented as geometric mean and standard deviation; dotted line for granulocyte counts marks the normal value; arrows indicate G-CSF injections: (a,b) granulocyte data (a), filgrastim plasma concentrations (data points) and corresponding pharmacokinetic (PK) model predictions (lines) for scheduling $4 \times 10 \mu\text{g}$ on consecutive days starting directly, 1 day or 2 days after cyclophosphamide application (b); (c,d) granulocyte data (c), filgrastim plasma concentrations (data points) and corresponding PK model predictions (lines) for scheduling $2 \times 20 \mu\text{g}$ on consecutive days starting directly, 1 or 2 days after cyclophosphamide application (d); (e,f) granulocyte data (e), pegfilgrastim plasma concentrations (data points) and corresponding PK model predictions (lines) for a single dose of $40 \mu\text{g}$ directly, 1 or 2 days after cyclophosphamide application (f).

Figure 3. Schematic structure of the pharmacokinetic model for filgrastim and pegfilgrastim. Compartments and model equations are explained in the text.

Table 1. Quantities of the pharmacokinetic (PK) model for both filgrastim and pegfilgrastim, type, unit and explanation of all quantities. Furthermore, PK parameter estimates are presented for both filgrastim and pegfilgrastim based on fitting the PK model to data. In parentheses are confidence intervals for all fitted parameters (see materials and methods section). Parameter estimates were compared between the drugs

Symbol	Type	Unit	Explanation	Fitted value for filgrastim	Fitted value for pegfilgrastim	Relation between parameter estimates
$C_{G-CSF}^{sc,j}$	Function of time to be calculated	μg	Contains of the sub-compartments of the subcutaneous tissue	—	—	—
C_{G-CSF}^{cent}	Function of time to be calculated	μg	Contain of the central compartment (plasma concentration)	—	—	—
C_{G-CSF}^{per}	Function of time to be calculated	μg	Contain of the peripheral compartment	—	—	—
$Rate$	Given function of time	$\mu\text{g/h}$	Applied dose per hour, the area of this function equals the total dose applied	—	—	—
t_{inj}	Constant	h	Injection time (not fitted)	1/6	1/6	—
N	Constant	—	Number of sub-compartments in subcutaneous tissue (not fitted)	5	5	—
k_{sc}	Parameter	1/h	Transition between subcutaneous sub-compartments	0.74 (0.68, 0.79)	0.32 (0.29, 0.35)	Greater for filgrastim
vol	Parameter	ml	Volume of distribution	44 (35, 51)	1.5 (1.3, 1.8)	Greater for Filgrastim
k_{12}	Parameter	1/h	Transition from central to peripheral compartment	2.3e-3 (1.6e-3, 3.1e-3)	0.85 (0.82, 0.90)	Less important for filgrastim than for pegfilgrastim
k_{21}	Parameter	1/h	Transition from peripheral to central compartment	0.057 (0.046, 0.077)	0.25 (0.24, 0.26)	
k_{el}^{cent}	Parameter	1/h	Unspecific (renal) elimination	0.33 (0.29, 0.36)	0.25 (0.24, 0.26)	Greater for filgrastim
k_s^{GRA}	Parameter	$\mu\text{g/h}$	Specific elimination via granulocytes (G-CSF receptors or neutrophil elastase)	6.2e-5 (4.6e-5, 7.6e-5)	6.0e-6 (5.5e-6, 6.5e-6)	See Fig. 4
k_m^{GRA}	Parameter	μg	Michaelis-Menten constant of specific elimination	0.82 (0.60, 1.24)	0.016 (0.013, 0.020)	
GRA	Given function of time	—	Number of circulating granulocytes in $10^6/\text{L}$	—	—	—

estimated maximum specific elimination is also lower for pegfilgrastim. Additionally, specific elimination of pegfilgrastim appeared to be saturated much more quickly than elimination of filgrastim. Corresponding Michaelis-Menten kinetics are displayed in Fig. 4. Finally, protein binding was estimated to be less important for filgrastim than for pegfilgrastim (lower transition coefficients for filgrastim).

Discussion

Here, we have constructed a biomathematical PK model for two pharmaceutical G-CSF derivatives (filgrastim and

pegfilgrastim) in healthy and in granulocytopaenic mice. The model fits well to data obtained by a series of mouse experiments with close meshed time series of G-CSF plasma concentration. Since the same model structure is used for both derivatives, differences in model parameters allow comparisons of important PK properties between the two drug derivatives. Additionally, the model allows prediction of the time course of the G-CSF derivatives when G-CSF scheduling, and the resulting granulocyte time courses are available. Thus, it is not fully data independent at this stage, but it is a first step towards a fully data independent combined PK and PD model, which is under development.

Figure 4. Comparison of estimated specific elimination between filgrastim and pegfilgrastim. Absolute amount of granulocyte colony-stimulating factor (G-CSF) eliminated by granulocytes. True elimination rate is obtained after multiplying specific elimination with the actual number of granulocytes in $10^6/L$. Elimination of pegfilgrastim is saturated for small amounts of G-CSF while filgrastim elimination is saturated later and is higher.

Understanding of PK properties of G-CSF and its derivatives is crucial in order to guarantee optimal G-CSF prophylaxis, in several chemotherapy situations. Because of the strong relationship between G-CSF-induced granulocytosis and chemotherapy-induced granulocytopenia, and the relationship between number of granulocytes and degradation of G-CSF, it is difficult to optimize G-CSF treatment empirically. Therefore, we aim to provide biomathematical models of granulopoiesis under G-CSF and chemotherapy in order to allow predictions about optimal G-CSF schedules.

The most frequently used pharmaceutical G-CSF derivatives are filgrastim and pegfilgrastim. In the last few years, there has been a clear clinical trend towards replacement of multiple filgrastim injections by a single pegfilgrastim injection. Nevertheless, we believe that filgrastim will not disappear completely from clinical practice as it can be applied more individually and flexibly, which can be specially important in the case of dose-dense chemotherapies with cycle duration of 14 days (40).

A first step of our modelling approach has been to construct this PK model of the derivatives filgrastim and pegfilgrastim in mice. The model is based on physiologically plausible assumptions that were translated into ordinary differential equations. It consists of three compartments that represent subcutaneous tissue in which the drugs were injected, the central compartment in which plasma concentration of the drug is measured, and a peripheral compartment of drugs characterizing reversible processes of temporary elimination of G-CSF from the central compartment (for example by protein binding; see Kuwabara *et al.*

(39)). Occurrence of maximum drug concentration in the blood is clearly delayed after subcutaneous injection, which could be caused, for example, by lymphatic absorption (41). Hence, transition between subcutaneous tissue and the central compartment has been modelled by five identical sub-compartments of the subcutaneous compartment, resulting in a gamma-like distribution of transit time (32). Transition between central and peripheral compartments is a two-way first-order kinetic representing reversibility of this process (42). The drug is irreversibly eliminated from the central compartment by two independent processes, non-specific elimination of the drug *via* renal clearance, modelled by a first-order kinetic (16,22,42,43) and specific degradation of the drug saturated by Michaelis-Menten kinetics and proportional to the number of circulating granulocytes (18,21,42,44–47). Other authors (for example Stute *et al.* (43)) proposed a pure first-order elimination kinetic of (non-glycosylated) G-CSF, which is a special case of Michaelis-Menten kinetics. However, confidence intervals of our model parameter estimates revealed a clearly saturated specific elimination kinetic. Specific degradation is mediated by G-CSF receptor binding and internalization (15,18,21,37,48,49) or neutrophil elastase (17,19,35,36). We assumed proportionality with respect to granulocytes since neutrophil elastase is mainly produced by neutrophils (17). Implicitly, we also assumed proportionality of G-CSF receptor-mediated clearance with the number of granulocytes, which might be only roughly correct because of a large number of G-CSF receptors in bone marrow and spleen (9,50), and different dynamic behaviour of bone marrow and circulating granulocytes during the course of therapy (31,32). Since time course of bone marrow is not available to that extent, this limitation of the model can only be resolved later by a full PK/PD model of granulopoiesis under G-CSF and chemotherapy, including the bone marrow dynamics. However, we will need additional experimental data, especially with respect to the effect of chemotherapy to bone marrow dynamics for such a model. As a consequence, we have to imprint the time course of granulocytes and can only predict the time course of G-CSF plasma concentrations at this stage of modelling.

Since the parameters of the model are unknown, we estimated them by fitting the model to experimental data. To obtain reliable estimates, especially with respect to the specific degradation kinetic, it is necessary to have close meshed time series data of granulocytes and G-CSF plasma concentrations under both granulocytotic and granulocytopenic conditions and under various G-CSF dosing and timing schedules as well. Although the application of G-CSF and its derivatives has been investigated extensively in the literature (e.g. 51–56, among others), we were unable to find appropriate published data that fulfil all of the requirements above. Therefore, it was

necessary to perform our own experiments designed under the viewpoint of model requirements.

We obtained a good agreement between model and data for all experimental results. We were able to characterize PK properties of filgrastim and pegfilgrastim on the basis of a small set of different model parameters. We estimated that filgrastim would be absorbed more quickly by the central compartment than pegfilgrastim, according to experimental results (23,57,58). Additionally, we estimated that filgrastim has a higher distribution volume and accordingly has lower protein binding affinity than pegfilgrastim. Elimination kinetics of filgrastim and pegfilgrastim has been analysed in detail. In agreement with results from the literature, we estimated that filgrastim has both higher non-specific elimination and higher specific degradation (25). The latter includes both higher saturation of pegfilgrastim elimination and higher maximum elimination of filgrastim as well. This might be due to reduced receptor binding affinity of pegylated G-CSF or hydrophilic properties of pegylation (58–61). Additional evidence for this mechanism has been collected by experiments with newly developed G-CSF derivative, Maxy-G34 (Maxygen Inc., Redwood City, CA, USA). Maxy-G34 has more pegylation groups compared to pegfilgrastim. With the same model approach presented in this paper, we were able to show that Maxy-G34 has specific degradation even lower than that for pegfilgrastim (62).

Our model as presented is not the first attempt to construct a PK model of filgrastim, pegfilgrastim or other G-CSF derivatives. Kuwabara *et al.* (42) proposed a PK model of lenograstim with the same degradation mechanisms, in order to estimate bioavailability. Sarkar and Lauffenburger (47) proposed a PK/PD model of G-CSF derivatives, including a model of G-CSF action at the cellular level. Wang *et al.* (63) developed a PK/PD model of filgrastim in healthy volunteers and Roskos *et al.* (64) constructed a similar PK/PD model of pegfilgrastim again for healthy volunteers. Yang *et al.* (25) constructed a PK model of filgrastim and pegfilgrastim application in healthy and nephrectomized rats without modelling the direct influence of granulocyte-mediated clearance and Vainstein *et al.* (65) proposed a PK/PD model of filgrastim application in healthy humans. Foley and Mackey (66) developed a theory for delay differential equations models of granulopoiesis under chemotherapy and G-CSF treatment. In an earlier study, we have constructed a PK/PD model of filgrastim in healthy and chemotherapy-treated humans (32), but so far, there is no PK model of both filgrastim and pegfilgrastim studied under granulocytotic and granulocytopenic conditions. In our opinion, the study of PK properties of filgrastim and pegfilgrastim under granulocytopenic conditions is crucial in order to

construct future models that can be used for therapy optimization. Additionally, in comparison to other models (25,63), our model is based on unique parameter setting which is constant for all doses and therapy schedules considered. In our opinion, this property is important in order to make predictions about schedules not yet tested.

Although physiologically plausible, concrete PK parameter estimates of our model would require validation by further experiments. As proof of principle we have been able to show that the preliminary human PK model of filgrastim proposed in Scholz *et al.* (32) could be translated to data of normal and cyclophosphamide-induced neutropenic mice, treated with either filgrastim or pegfilgrastim, in different schedules. Further validation of this translational approach will be provided by modelling data from rats treated with pegfilgrastim or Maxy-G34 (62). Therefore, the model structure presented here was not derived by formal mathematical optimization criteria but by both physiological assumptions and former or ongoing modelling work, in other species or other applications as well.

In the next step of our work, we plan to develop a full PK and PD model in the murine situation, which specially accounts for bone marrow effects of combined G-CSF and chemotherapy treatment. Therefore, a series of experiments will be performed to generate the data required for modelling (especially time series of bone marrow changes during chemotherapy, with or without G-CSF stimulation). In our opinion, it is necessary to construct a rigorous PK/PD model in the mouse situation first, for which bone marrow data are experimentally available and to translate the model later to the human situation for which only clinical data of peripheral cell counts are available. We aim to translate the murine model to the human situation later by keeping identified model mechanisms constant but changing model parameters for which the present paper is the proof of principle.

The resulting models will allow quantitative predictions about time course of bone marrow cell stages, circulating blood cells and cytokine concentrations for chemotherapeutic regimens not yet tested. Systematic model simulations of variable therapy parameters can lead to predictions concerning, for example, optimal application schedules of haematopoietic growth factors. Thus, the present paper is only a step in our systems-biology approach to construct a rigorous model of granulopoiesis under chemotherapy and G-CSF application, which allows optimization of G-CSF therapy, by systematic model simulations.

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