Pharmacokinetic/Pharmacodynamic Analysis of Neutrophil Proliferation Induced by rhG-CSF in Patients Receiving Antineoplastic Drugs

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In the present study, we analyzed the effect of recombinant human granulocyte colony-stimulating factor (rhG-CSF) on neutrophil counts in cancer patients undergoing chemotherapy using a previously developed pharmacokinetic/pharmacodynamic model.7 The time profiles of neutrophil counts in blood after repeated administration of rhG-CSF to lung cancer patients undergoing chemotherapy could be analyzed by this model by considering the inhibition of neutrophil production by antineoplastic drugs. Although deviation was observed between the predicted and observed neutrophil counts in ovarian cancer patients, it may be possible to use this model for determining a rational dosage regimen of rhG-CSF for patients undergoing chemotherapy.

Key words—granulocyte colony-stimulating factor; pharmacodynamic model; dosage regimen; chemotherapy; neutrophil

INTRODUCTION
Recombinant human granulocyte colony-stimulating factor (rhG-CSF) is widely used for the treatment of neutropenia associated with cancer chemotherapy, leukemia and aplastic anemia.2-5 Although its mechanism for disposition has been studied extensively in rodents,6 the establishment of a pharmacokinetic/pharmacodynamic model is needed to predict the pharmacological effect of rhG-CSF in humans. We developed such a model in our previous study and showed that the model enables prediction of increase in the number of neutrophils after administration of rhG-CSF to healthy volunteers.7 In this model, the pharmacological action of rhG-CSF was assumed to be proportional to the amount of a ternary complex composed of rhG-CSF, its receptor and the putative effector molecule.7 The GTP-binding protein may be responsible for this putative effector molecule, since the binding of rhG-CSF to the receptor induces the activation of adenylate cyclase through the GTP-binding protein. By using this model, we were able to obtain time profiles of the increase in the number of neutrophils after administration of rhG-CSF.7 We also provided a theoretical background for the fact that the increase in neutrophil count after s.c. administration is larger than that after i.v. administration of the same dose.7

However, for rhG-CSF treatment of patients who have received antineoplastic drugs, it is essential to consider the decrease in the number of neutrophils and their precursor (C-CFU) in analyzing the effect of rhG-CSF. In the present study, we refined our previous model by considering the inhibition of neutrophil production caused by exposure to antineoplastic drugs in order to establish a rational regimen for rhG-CSF treatment.

METHODS

Patients’ Backgrounds Time profiles of neutrophil counts in 3 female patients with lung cancer and 3 female patients with ovarian cancer were analyzed in the present study. Two of the lung cancer patients received the i.v. administration of paclitaxel (200 mg/m²) and carboplatin, whereas one of the lung cancer patients received the i.v. administration of paclitaxel (60 mg/m²) and cisplatin (80 mg/m²). The dose of carboplatin was determined so that the AUC of this drug will be 6 and 5mg/ml•min for the ovarian and lung cancer patients, respectively, according to the Calvert’s equation.8 Two of the ovarian cancer patients received the i.v. administration of paclitaxel (100 mg/m²), cisplatin (75 mg/m²) and adriamycin (40 mg/m²), whereas one of the ovarian...
cancer patients received the i.v. administration of paclitaxel (175 mg/m²) and carboplatin. The lung cancer patients received the same s.c. dose of rhG-CSF (filgrastim) 8 days after initiation of chemotherapy, whereas the ovarian cancer patients received s.c. administration of rhG-CSF (filgrastim) at a dose of 75 mg/kg day 5 after initiation of chemotherapy. Time profiles of the neutrophil counts in the blood of these patients were determined.

**Analysis of the Effect of rhG-CSF Using a Pharmacokinetic/Pharmacodynamic Model**

The pharmacokinetic/pharmacodynamic model used for the analysis of neutrophil counts after administration of rhG-CSF was a modification of the previously described model (Fig. 1). In this model, it is assumed that the specific binding of rhG-CSF to the receptor on C-CFU induces the activation of adenylate cyclase through the GTP-binding protein, leading to the proliferation of neutrophils. The equilibrium of rhG-CSF ([D] (pM), receptor [R] (sites/cell) and effector [E] (sites/cell) in a steady state is given by Eqs. (1) and (2).

\[
[D] 
\begin{align*}
\text{Plasma} & \\
\text{rhG-CSF} & \\
\text{Kd} & \\
\text{Receptor} & \\
\text{[R]} & \\
\text{G-CSF-receptor complex} & \\
\text{DR} & \\
\text{Kd} & \\
\text{Effector} & \\
\text{[E]} & \\
\text{G-CSF-receptor-effector complex} & \\
\text{DRE} & \\
\end{align*}
\]

\[
\frac{[D] 
\begin{align*}
\text{Plasma} & \\
\text{rhG-CSF} & \\
\text{Kd} & \\
\text{Receptor} & \\
\text{[R]} & \\
\text{G-CSF-receptor complex} & \\
\text{DR} & \\
\text{Kd} & \\
\text{Effector} & \\
\text{[E]} & \\
\text{G-CSF-receptor-effector complex} & \\
\text{DRE} & \\
\end{align*}
\]

where \([DR] \) (sites/cell) and \([DRE] \) (sites/cell) are the concentrations of G-CSF-receptor complex and rhG-CSF-receptor-effector ternary complex, respectively, and \(K_{d1} \) (pM) and \(K_{d2} \) (sites/cell) are the dissociation constants of \([DR] \) and \([DRE] \), respectively.

The concentration of rhG-CSF-receptor-effector ternary complex \([DRE] \) is assumed to be proportional to the neutrophil proliferative activity. The receptor occupancy (%) by rhG-CSF is described by Eq. (3).

\[
\Phi = \frac{[D] + [DRE]}{R_0}
\]

where \(R_0 \) represents the total concentration of receptors (sites/cell).

Then the relationship between \([D] \) and \([DRE] \) is derived from Eqs. (1)–(3) as follows:

\[
[DRE] = \left(\frac{b - \sqrt{b^2 - 4R_0E_0}}{2}\right)
\]

\[
b = K_{d2} \cdot \frac{([D] + [DRE])}{[D] + R_0 + E_0}
\]

where \(E_0 \) represents the total concentration of effectors (sites/cell).

When \([D] \) increases to infinity, \([DRE] \) approaches the maximum value of \([DRE_{max}] \), which is given by Eq. (6):

\[
[DRE_{max}] = \frac{(K_{d2} + R_0 + E_0)^2}{\sqrt{((K_{d2} + R_0 + E_0)^2 - 4R_0E_0)}}
\]

Assuming that the pharmacological effect of rhG-CSF is in proportion to \([DRE] \), the normalized relative effect of rhG-CSF is given by Eq. (7).

\[
K_s \cdot N_{C-CFU, SS} = \frac{K_{d1 \cdot \text{max} \cdot [DRE]}}{[DRE_{max}] \cdot N_{C-CFU, SS}}
\]

\[
K_s = K_{d1 \cdot \text{max} \cdot \left(\frac{b - \sqrt{b^2 - 4R_0E_0}}{2}\right)}{\left(\frac{K_{d2} + R_0 + E_0}{\sqrt{((K_{d2} + R_0 + E_0)^2 - 4R_0E_0)}}\right)}
\]

where \(K_s \) (h⁻¹) is the rate constant for the generation of neutrophils from C-CFU, which is the precursor of neutrophils, and \(K_{d1 \cdot \text{max}} \) (h⁻¹) is the maximum

![Fig. 1. Pharmacokinetic/Pharmacodynamics of rhG-CSF](image-url)

The rhG-CSF in blood binds to its receptor to form the drug–receptor complex \([DR] \), and then this complex associates with an effector molecule to form a ternary complex \([DRE] \). Under physiological conditions, neutrophils are generated from C-CFU with the rate constant of \(K_s \) and decomposed with the rate constant of \(K_s \). Generation of neutrophils from C-CFU is accelerated in the presence of the ternary complex \([DRE] \). In the presence of antineoplastic drugs, it is assumed that the number of C-CFU is given by \(1 - \exp(-K_s \cdot t) + \exp(-K_s \cdot t) \cdot \Phi \). See the text for details.
value of $N_0$, $N_{C-CFU,SS}$ (cells/ml) represents the number of C-CFU in a steady state.

In the absence of rhG-CSF, the rate of generation of neutrophils is assumed to be constant with the rate of $K \cdot N_{C-CFU}$ (cells/ml/h), and the decomposition of blood neutrophils follows a first-order elimination process at the rate constant of $K$ (h$^{-1}$). Based on this assumption, the neutrophil count in blood at time $t$ ($N$(cells/ml)) is given by Eq. (8):

$$dN/dt = K \cdot N_{C-CFU,SS} - K \cdot N\quad(8)$$

The constant value of $N_0$ (cells/ml), which represents the net neutrophil count in blood, should be maintained (Eq. (9)):

$$N_0 = K \cdot N_{C-CFU,SS}/K\quad(9)$$

In the presence of rhG-CSF, the rate of generation of neutrophils is increased with the rate constant of $K_s$ (h$^{-1}$) as follows:

$$dN/dt = K \cdot N_{C-CFU,SS} + K_s \cdot N_{C-CFU,SS} - K \cdot N\quad(10)$$

Then, $N_0$ (cells/ml) is mean neutrophil count in blood for patients in the evening of the day before administration of an antineoplastic drug.

$$dN/dt = N_0 \cdot K_s + K_s \cdot N_{C-CFU,SS} - K \cdot N\quad(11)$$

After administration of an antineoplastic drug, it is assumed that $N_{C-CFU}$ is given by

$$N_{C-CFU} = N_{C-CFU,SS} \cdot (1 - \exp (-K_s \cdot t)) + \exp (-K_s \cdot t)\quad(12)$$

and, consequently, the number of neutrophils is given by Eq. (13):

$$dN/dt = (N_0 \cdot K_s + K_s) \cdot N_{C-CFU,SS} \cdot (1 - \exp (-K_s \cdot t)) + \exp (-K_s \cdot t)) - K \cdot N\quad(13)$$

In Eqs. (12) and (13), $K_s$ (h$^{-1}$) represents the first order rate constant for the decrease in the neutrophil number induced by antineoplastic drugs, and $K_s$ (h$^{-1}$) represents the first order rate constant for the recovery of the neutrophil number. In general, the number of neutrophils after repeated administration of rhG-CSF for $n$ (n$\geq$2) times is given by Eq. (14):

$$dN/dt = N_{n-1} + (N_0 \cdot K_s + K_s) \cdot N_{C-CFU,SS} \cdot (1 - \exp (-K_s \cdot t)) + \exp (-K_s \cdot t)) - K \cdot N_n\quad(14)$$

Time profiles of neutrophil counts were fitted to Eqs. (13) and (14) to obtain $K_s$ and $K_s$ values using a program reported previously.9 In the fitting, the initial value of $N_0$ was set to be equal to the neutrophil number calculated for the time just prior to $n$th administration of rhG-CSF. The values of $K_s$, $N_{C-CFU,SS}$ and $K_s$ were fixed to 2.12 (cell/ml/h) 0.107 (hr$^{-1}$), respectively, according to our previous report.9

RESULTS

Time Profiles of Neutrophil Counts in Patients

Time profiles of neutrophil counts in patients with lung cancer and ovarian cancer are shown in Figs. 2 and 3, respectively. These profiles were fitted to Eq.

**Fig. 2.** Time Profiles of Neutrophil Counts in Blood from Three Patients with Lung Cancer

Five days after initiation of chemotherapy, the lung cancer patients started receiving rhG-CSF (filgrastim) by s.c. administration at a dose of 75 µg/body/day for 8 consecutive days. Each point and vertical bar represents the mean ± S.D. of three determinations. The solid line represents the fitted line. The dotted line represents the predicted time profile for neutrophil number, which was calculated on the basis of the assumption that these patients did not receive rhG-CSF (filgrastim).
Eight days after initiation of chemotherapy, the ovarian cancer patients started receiving rhG-CSF (filgrastim) by s.c. administration at a dose of 75 μg/body/day for 5 consecutive days. Each point and vertical bar represents the mean ± S.D. of three determinations. The solid line represents the fitted line. The dotted line represents the predicted time profile for neutrophil number, which was calculated on the basis of the assumption that these patients did not receive rhG-CSF (filgrastim).

Time profiles of neutrophil counts in blood were simulated assuming that the neutrophil number is $3 \times 10^3/\mu l$ blood prior to the initiation of chemotherapy. Time profiles were predicted for $K_a$ of 0.015, 0.020 and 0.025 hr$^{-1}$. The fitted lines are also shown in Figs. 2 and 3. The fitted line almost superimposed on the data. The calculated $K_a$ and $K_e$ values were $0.0145\pm0.0002$ (hr$^{-1}$) and $0.000834\pm0.000176$ (hr$^{-1}$) for lung cancer patients, and $0.0210\pm0.0002$ (hr$^{-1}$) and $0.000970\pm0.000222$ (hr$^{-1}$) for ovarian cancer patients respectively.

Time profiles of neutrophil counts were also simulated by assuming that the neutrophil number before initiation of the administration is $3\times10^3$ and $4.5\times10^3$.
Fig. 5. Prediction of Neutrophil Number after Administration of rhG-CSF

Time profiles of neutrophil counts in blood were simulated assuming that the neutrophil number is $4.5 \times 10^3$ /mL blood prior to the initiation of chemotherapy. Time profiles were predicted for $K_a^*$ of 0.015, 0.020 and 0.025 hr$^{-1}$.

neutrophils/mL blood, respectively (Figs. 4 and 5). For this simulation, $K_a^*$ of 0.015, 0.020 and 0.025 hr$^{-1}$ and $K_e^*$ of 0.00097 hr$^{-1}$ were used.

**DISCUSSION**

Neutropenia is one of the major obstacles for continuation of tumor therapy with antineoplastic drugs.$^{10,11}$ After chemotherapy, rhG-CSF is widely used to increase the number of neutrophils. An accurate pharmacokinetic/pharmacodynamic model of rhG-CSF is needed for determining a rational dosage regimen of rhG-CSF.$^7$ In the present study, we incorporated decrease in the number of neutrophils in our previously described pharmacokinetic/pharmacodynamic model of rhG-CSF.$^7$

In the present analysis, we assumed that the number of neutrophils after administration of antineoplastic drugs is given by Eq. (11). This assumption is based on the fact that the number of neutrophils is reduced after administration of antineoplastic drugs and later recovers to the normal level.$^{12,13}$ In a strict manner, such time profiles for the neutrophil count should be described by considering the pharmacological effect of antineoplastic drugs on C-CFU, along with the rate constants for the proliferation and differentiation of C-CFU and that for the turnover of neutrophils as described previously.$^{14-16}$ From such a point of view, the values of $K_a^*$ and $K_e^*$ should depend on the kind of antineoplastic drugs and their plasma concentration profiles.$^{14-16}$ Under conditions in which proliferation and differentiation of C-CFU are almost completely inhibited by antineoplastic drugs, the $K_a^*$ value should represent the rate constant for the turnover of neutrophils. Since antineoplastic drugs are administered at their maximum tolerated doses, it is plausible that the calculated $K_a^*$ values is consistent with the turnover rate of neutrophils.

By using the present model, the time profiles of neutrophil counts could be analyzed for patients with lung cancer, whereas deviation was observed between the predicted and observed neutrophil counts in ovarian cancer patients (Figs. 2 and 3). It may be difficult to analyze the neutrophil number if the damage of progenitor cells induced by the chemotherapy is extensive. For these patients, we also simulated the neutrophil counts assuming that they did not receive rhG-CSF. As shown by the dotted lines in Figs. 2 and 3, it was demonstrated that the recovery of neutrophil counts is greatly delayed without the administration of rhG-CSF.

Although results of analysis using many patients are required for validation of this model, it may be possible to use this model in order to determine a rational dosage regimen of rhG-CSF. For this purpose, we performed simulation of neutrophil counts after...
administration of antineoplastic drugs and rhG-CSF (Figs. 4 and 5). Assuming that a patient had $4.5 \times 10^9$ neutrophils/$\mu l$ blood before the administration of antineoplastic drugs, the time profiles of neutrophil count was simulated as a function of $K_a^*$ value (Fig. 5). In order to maintain a minimal neutrophil count in blood ($0.5 \times 10^9$ neutrophils/$\mu l$ blood), the patient should receive rhG-CSF 9 days after the initiation of chemotherapy if the $K_a^*$ value is 0.015 hr$^{-1}$, which was determined for patients undergoing chemotherapy for lung cancer (Fig. 3). However, these patients should receive rhG-CSF 6 days after initiation of chemotherapy if the $K_a^*$ value is 0.02 hr$^{-1}$, which was calculated for patients undergoing chemotherapy for ovarian cancer (Fig. 2). In addition, if it is assumed that the patient has $3.0 \times 10^9$ neutrophils/$\mu l$ blood before the administration of antineoplastic drugs and that the $K_a^*$ value is 0.015 hr$^{-1}$, rhG-CSF should be administered 7 days after initiation of the chemotherapy, in order to maintain the minimal level of neutrophils (Fig. 4). In contrast, rhG-CSF should be administered 4 days after the initiation of chemotherapy if the $K_a^*$ value is 0.02 hr$^{-1}$ (Fig. 2). It is suggested that we can provide a rational dosage design of rhG-CSF by analyzing the decline of neutrophil count in blood after initiation of chemotherapy.

In the present study, we found that the effect of rhG-CSF on neutrophil count in patients undergoing chemotherapy can be analyzed by using the pharmacokinetic/pharmacodynamic model. Although results of analysis using many patients is required, it may be possible to use this model for the purpose of determining the rational regimen of rhG-CSF for patients undergoing chemotherapy.

REFERENCES AND NOTES
