Pharmacodynamic Modeling of the Entire Time Course of Leukopenia after a 3-Hour Infusion of Paclitaxel

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The entire time course of leukopenia after anticancer treatment is clinically more relevant than a singly measured nadir count. In order to identify factors associated with neutropenic fever, a mechanistic pharmacodynamic model with two compartments corresponding to leukocytes in bone marrow and peripheral blood was applied to describe the time course of leukopenia. Seventeen patients with breast cancer were treated with 210 mg/m^2 of paclitaxel infused over 3 h as a single agent in a phase II study. Adequate fitting of the time course of leukopenia was achieved in all patients, and time-dependent parameters, including the time period during which leukocyte counts remained below 2000/μl and the area between the curve for time versus leukocyte counts and the line of a leukocyte count of 2000/μl ($A<2000$), were calculated in each patient. Leukopenia was not significantly correlated with pharmacokinetic parameters, including time above a threshold concentration or the area under the time-concentration curve. A negative correlation between age and the sensitivity parameter of the pharmacodynamic model was observed ($r^2=0.21$, $P=0.07$). Patients who experienced neutropenic fever had a larger $A<2000$ than patients who did not experience fever (4512 vs. 6 days/μl, $P=0.05$), but fever was not significantly related to any pharmacokinetic parameter or the leukocyte nadir count. Febrile episodes were better associated with the time course of leukopenia than the singly measured nadir count, and the pharmacodynamic model presents a novel platform to analyze the entire time course of leukopenia.

Key words: Pharmacokinetics — Pharmacodynamics — Leukopenia — Fever — Chemotherapy

Leukopenia is the primary toxicity of many anticancer agents and precludes dose escalation or scheduled administration of repeated cycles. Most pharmacodynamic analyses of anticancer agents focus on this toxicity, and a singly measured nadir leukocyte count or the surviving fraction of leukocytes at the nadir (nadir count divided by the pretreatment count) is modeled.[1–4] However, leukopenia changes continuously with time after treatment, and the singly measured nadir count is not the best predictor of clinically relevant events, including fever or toxic death, considering that patients with prolonged leukopenia have a greater risk of infection than patients with more rapid recovery.[5] The time period during which the leukocyte count remains below a certain level (i.e., 1000 or 2000/μl, Fig. 1a) or the area between the curve of time versus leukocyte counts and the line of a specific leukocyte count (Fig. 1b) may be clinically more important than the singly measured nadir count (Fig. 1c). Not only the magnitude of leukopenia, but also the time course of leukopenia should be considered in pharmacodynamic analysis of anticancer agents.

In most of the conventional pharmacodynamic analyses, information on plasma concentrations of the drug, which change with time, is summarized by a time-independent parameter such as the area under the time-concentration curve (AUC), the peak concentration, or the time above a threshold concentration.[2, 6–10] The relationship between one of these summary parameters (e.g., AUC) and the nadir count, which is also a summary parameter of pharmacodynamics, is evaluated by using a model. In this case, patients with the same AUC but a different time course of plasma concentrations would have the same nadir value, although they have a different time course of leukopenia. It is useful to consider the time course of the drug concentration when the time course of leukopenia after treatment with an anticancer drug is modeled. Although pharmacodynamic models for the time course of leukopenia have been reported, they were mathematical and ignored the time course of the drug concentration.[11–14]

Recently we developed a model to describe the entire time course of leukopenia after treatment with anticancer agents by using the time course of plasma concentrations as input into the model.[15] By using the model, the time that leukocyte counts remain below a certain value or the area between the time course of leukopenia and the line of a specific leukocyte count can be estimated. In this study, we have applied the pharmacodynamic model to the time course of leukopenia after a 3-h infusion of paclitaxel in patients with breast cancer who were treated in a phase II
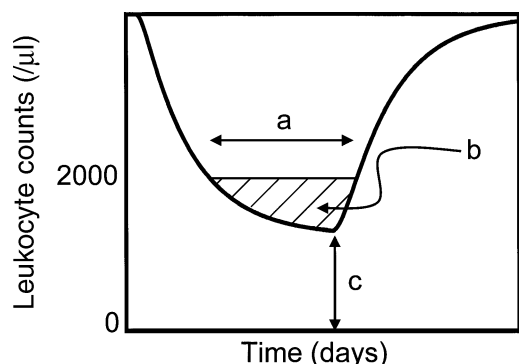
Fig. 1. Clinically relevant parameters of leukopenia. The time period during which the leukocyte count remains below a certain level, e.g. 2000/μl (a), or the area between the curve of time versus leukocyte counts and the line of a leukocyte count of 2000/μl (b) may be clinically more important than the singly measured nadir count (c).

Table I. Characteristics of Patients with Breast Cancer Treated in a Phase II Study of Paclitaxel Infused over 3 h

Number of patients	17
Age (years)	33–68 (median 50)
Sex (female/male)	17/0
Performance status (0/1/2)	9/7/1
Body surface area (m^2)	1.46±0.08
Previous myelosuppressive chemotherapy (yes/no)	14/3
Previous radiotherapy (yes/no)	5/12
Total protein (g/dl)	6.8±0.6
Albumin (g/dl)	3.8±0.2
Total bilirubin (mg/dl)	0.7±0.2
Creatinine (mg/dl)	0.6±0.1

study of paclitaxel as a single agent. The purposes of this study were to evaluate the applicability of the model to these patients, to evaluate the relationship between patient characteristics and the time course of leukopenia, and to identify pharmacodynamic parameters related to neutropenic fever.

PATIENTS AND METHODS

Patients The data used in this study were obtained from a phase II study of paclitaxel at a dose of 210 mg/m^2 infused over 3 h against advanced or metastatic breast cancer.[16] We used data from patients who underwent optional blood sampling for pharmacokinetic evaluation. In order to obtain adequate fitting of the time course of leukopenia, we excluded data from patients whose leukocyte counts were not measured more than once a week. Of 62 patients treated in the phase II study, 24 underwent pharmacokinetic blood sampling, but seven of these did not have leukocyte counts measured more than once a week. Data from the remaining 17 patients were used in this study (Table I). The number of leukocyte counts measured in 3 weeks after chemotherapy ranged from 5 to 9 with the median of 7. All patients had adequate organ function, and 14 patients had been treated with myelosuppresive chemotherapy but had recovered fully from the toxicities of the previous treatment at the time of paclitaxel administration. The number of previous regimens of myelosuppresive chemotherapy ranged from 0 to 2, with a median of 1. None of the patients was treated with a granulocyte colony-stimulating factor in the phase II study of paclitaxel. The study was approved by the Institutional Review Board and all patients gave informed consent.

Pharmacokinetic study For pharmacokinetic evaluation, heparinized blood samples were collected at 1.5 h into and at the end of infusion, and at 5, 15, 30 min and 1, 2, 3, 4, 6, 12, 24 and 48 h after the end of infusion of the first cycle. Plasma concentrations of paclitaxel were determined by HPLC.[10] A drug concentration versus time curve in each patient was fitted by using a linear 3-compartment model. A nonlinear pharmacokinetic model with Michaelis-Menten kinetics at distribution and elimination processes was suggested for paclitaxel.[7, 17] However, we used the linear model because the difference in the AUC between the nonlinear and the linear models was minimal and because the linear model is easier than the nonlinear model to handle in pharmacodynamic analysis.[15]

Pharmacodynamic study The entire time course of leukopenia after treatment with paclitaxel was fitted in each patient by using a pharmacodynamic model which we have recently developed.[15] Briefly, the pharmacodynamic model is an indirect response model[18] and has two compartments corresponding to bone marrow (Z_1), where leukocytes are produced, and peripheral blood (Z_2), where leukocyte counts are measured. The model incorporates the fractional inhibition (F) of the rate of leukocyte production in bone marrow (k_1) as a function of drug exposure ($AUC(t)$), and linear kinetics for leukocyte movement from bone marrow to peripheral blood (k_2) and for the disappearance of leukocytes from peripheral blood (k_3). The IC in the model was a constant and corresponded to an AUC value that gave 50% inhibition of k_1 (Fig. 2). Hematopoietic stem cells are relatively resistant to chemotherapeutic agents or radiation because their generative rate is slow.[19] Therefore, it is hypothesized that stem cells are totally insensitive to anticancer agents in the model and myeloid cells are considered to be sensitive while they are in mitotic stages consisting of myeloblasts to myelocytes.[20] The inhibition of leukocyte production in bone marrow is assumed to be controlled by the exposure of

myeloid cells to anticancer drugs while they are in these sensitive cell stages (AUC(t)). T_s is the duration of these sensitive periods. The early phase of the inhibition of leukocyte production after chemotherapy is caused by drug exposure of myeloid cells that are in sensitive stages at the time of drug administration. Drug exposure till they get out of the sensitive stages is effective for inhibition (Fig. 2, $t<T_s$). For myeloid cells at the stem cell stage insensitive to anticancer drugs at the time of the dosage, drug exposure after they reach the sensitive stage and until they get out of the sensitive stage should contribute to the inhibition of leukocyte production. The effect of these cells on the leukocyte counts should be noted at a later phase (Fig. 2, $t \geq T_s$). Therefore, AUC(t) is a function of time (t), and an E_{max} model as a function of AUC(t) is used for the inhibition of leukocyte production in bone marrow. These drug exposures (AUC(t)) were calculated by using parameters of the 3-compartment pharmacokinetic model in each patient.
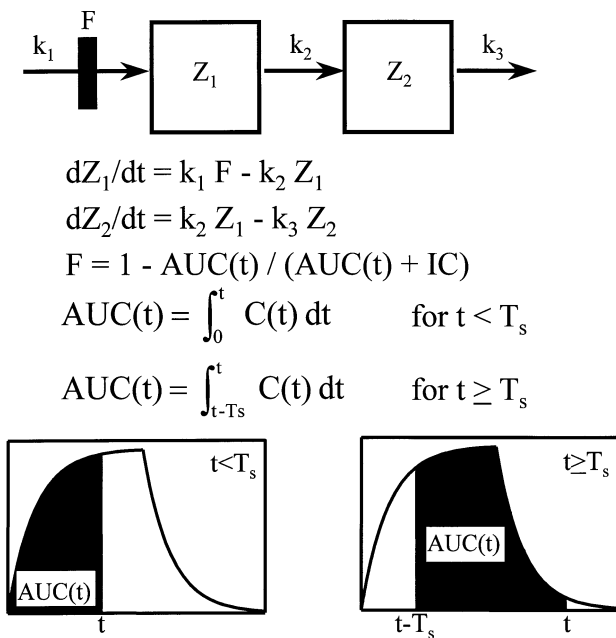
Leukocyte counts after treatment were divided by the pretreatment count, and the change of this surviving fraction was modeled. Therefore, the baseline value of Z_2 (peripheral leukocytes) is 1, and the baseline value of Z_1 is the compartment size of leukocytes in bone marrow relative to peripheral blood and set to A, which is estimated by fitting the observed leukocyte data. The half-life of leukocytes in peripheral blood is reported to be 7 h,[21-23] which gives $k_3=0.693/7=0.099$/h. At the baseline, leukocyte counts in bone marrow and peripheral blood are constant ($dZ_1/dt=dZ_2/dt=0$), and $k_1=k_2A=0.099$/h. In fitting the data of the surviving fraction of leukocytes, the lag-time (T_{lag}) before peripheral leukocyte counts begin to decrease is used. Four parameters, IC, T_s, A and T_{lag}, were estimated by using a nonlinear least-square regression program, WinNonlin (Pharsight Corporation, Cary, NC). The time course of leukocyte counts was calculated by multiplying

$$dZ_1/dt = k_1 F - k_2 Z_1$$

$$dZ_2/dt = k_2 Z_1 - k_3 Z_2$$

$$F = 1 - AUC(t) / (AUC(t) + IC)$$

$$AUC(t) = \int_0^t C(t)\,dt \qquad \text{for } t < T_s$$

$$AUC(t) = \int_{t-Ts}^t C(t)\,dt \qquad \text{for } t \geq T_s$$

Fig. 2. An indirect response model for pharmacodynamic analysis of the time course of leukopenia after chemotherapy. Z_1 and Z_2 are compartments of the model and correspond to leukocytes in bone marrow and peripheral blood, respectively. k_1, k_2, and k_3 are constants and F is the fractional inhibition of the rate of leukocyte production in bone marrow (k_1) as a function of drug exposure of myeloid cells during the sensitive stage (AUC(t)). T_s is the duration of the sensitive stage. For $t>T_s$, drug exposure of myeloid cells is effective for the inhibition of leukocyte production after myeloid cells reach the sensitive stage ($t-T_s$) and until they get out of the sensitive stage (t). IC is a parameter corresponding to the drug exposure causing 50% of the maximal inhibition.
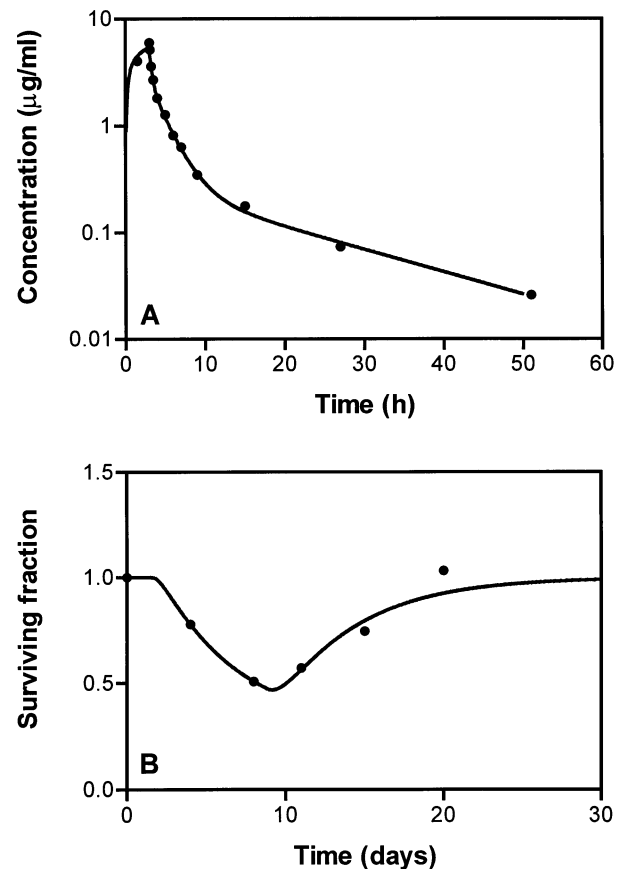

Fig. 3. Plots of observed concentrations of paclitaxel after a 3-h infusion and a concentration versus time curve predicted by the linear 3-compartment pharmacokinetic model (A), and plots of observed surviving fractions of leukocytes and the time course of the surviving fraction predicted by the pharmacodynamic model (B) in a representative patient.

233

the predicted time course of the surviving fraction by the pretreatment count.

Statistical analysis Relationships among patient characteristics, pharmacokinetic parameters, and pharmacodynamic parameters were evaluated with Pearson's correlation, and the difference of these parameters between patients who did and did not experience neutropenic fever was tested by using the Mann-Whitney U test. The Wilcoxon signed-rank test was used for paired comparison of measured nadir counts (or nadir time) and model-predicted nadir counts (or nadir time) in each patient.

RESULTS

Plasma concentration data in each patient were adequately fitted with a linear 3-compartment model, although minor underestimation of high concentrations around the end of infusion in some patients was observed. In fitting the time course of the surviving fraction of leukocytes by using the pharmacodynamic model, good agreement between the observed and predicted surviving fraction was obtained in each patient. Plots of observed and predicted time course of the drug concentration and the leukocyte surviving fraction in a representative patient are shown in Fig. 3. Residuals of prediction for the leukocyte surviving fraction (predicted value−observed value) of all measured surviving fractions in all patients were minimal, especially around the nadir (Fig. 4). A negative residual on the second day was caused by transient leukocytosis after paclitaxel administration with steroid premedication. Negative residuals after recovery from the nadir (13 to 21 days) were explained by the overshoot of leukocyte counts observed in some patients. Pharmacokinetic and pharmacodynamic parameters were successfully esti-
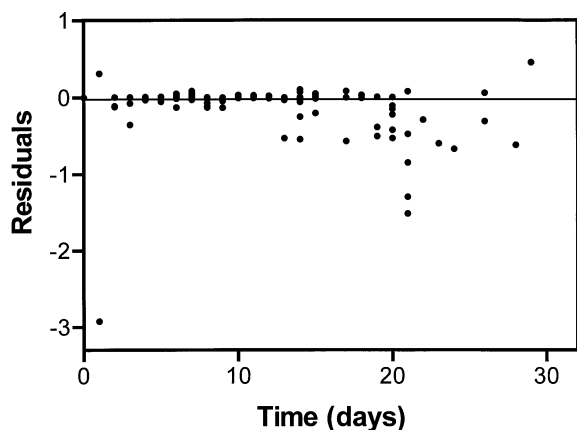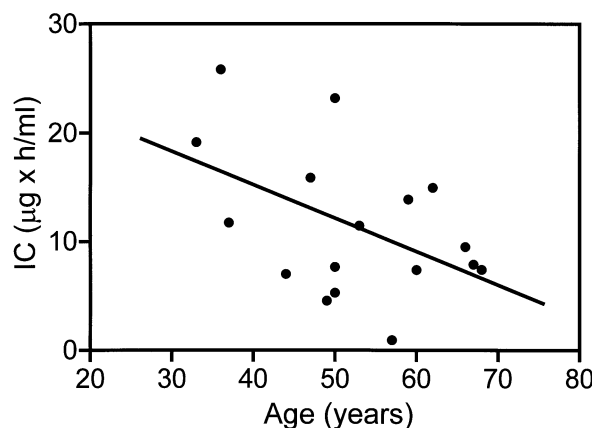

Fig. 4. Residuals (predicted value−observed value) of prediction for surviving fraction of leukocytes versus time after the treatment by a 3-h infusion of paclitaxel. Good agreement of predicted values and observed values was obtained for leukopenic periods.

Fig. 5. Scatter plots between age and a sensitive parameter of the pharmacodynamic model (*IC*). A negative correlation was observed ($r^2=0.21$, $P=0.07$).

Table II. Pharmacokinetic and Pharmacodynamic Parameters of Paclitaxel Infused over 3 h in Patients with Breast Cancer

	Pharmacokinetic parameters				Pharmacodynamic parameters			
	AUC (μg×h/ml)	CL (liter/h/m^2)	$T_{>0.1\,\mu M}$ (h)	$T_{>0.05\,\mu M}$ (h)	A	IC (μg×h/ml)	T_{lag} (h)	T_s (h)
mean±SD	23.8±7.1	9.7±4.8	23.6±4.8	37.8±6.7	4.3±3.3	11.4±6.7	61.2±25.8	219±76
median	22.3	9.3	23.3	37.2	2.9	9.5	55.9	209
range	10.2–40.2	5.2–20.6	16.4–35.8	26.0–53.2	0.4–11.9	0.9–25.8	5.4–107.9	115–438

A linear 3-compartment pharmacokinetic model and the pharmacodynamic model shown in Fig. 2 was used for the analysis of time vs. concentration data and the time course of leukocyte counts, respectively. Parameters were estimated by fitting the models to observed data in each patient.

AUC, area under the time versus concentration curve; CL, clearance; $T_{>0.1\,\mu M}$, time above concentration of 0.1 μM; $T_{>0.05\,\mu M}$, time above concentration of 0.05 μM; A, compartment size of leukocytes in bone marrow relative to peripheral blood before treatment; IC, the drug exposure of myeloid cells during the sensitive stages providing 50% of the maximum inhibition of leukocyte production; T_{lag}, lag time; T_s, duration of sensitive stages of myeloid cells.

Table III. Model-predicted Parameters of Leukopenia after 3-h Infusion of Paclitaxel

	Nadir count (/μl)	SF	Nadir time (days)	$A_{<4000}$ ($\times 10^3$ days/μl)	$A_{<2000}$ ($\times 10^3$ days/μl)	$T_{<4000}$ (days)	$T_{<2000}$ (days)
mean±SD	2000±900	0.34±0.12	11.7±2.0	14.7±8.3	1.6±2.3	10.3±4.2	3.0±3.3
median	1900	0.33	11.4	16.4	0.08	10.5	1.4
range	800−4100	0.15−0.59	9.1−17.3	0−26.5	0−6.7	0−18	0−8.3

The pharmacodynamic model shown in Fig. 2 was used to predict parameters of leukopenia after treatment in each patient. SF, surviving fraction of leukocytes (nadir count/pretreatment count); $A_{<4000}$, area between the curve of time versus leukocyte counts and the line of leukocyte counts of 4000/μl; $A_{<2000}$, area between the curve of time versus leukocyte counts and the line of leukocyte counts of 2000/μl; $T_{<4000}$, time that leukocyte counts remain below 4000/μl; $T_{<2000}$, time that leukocyte counts remain below 2000/μl.

Table IV. Comparison of Pharmacokinetics and Pharmacodynamics between Patients Who Did and Did Not Develop Fever

	With fever ($n=3$)	Without fever ($n=14$)	
AUC	15.9, 30.5, 40.2 [a]	10.2−32.6 (22.3) [b]	(μg×h/ml)
$T_{>0.1 \mu M}$	23.3, 31.7, 35.8	16.4−27.4 (22.4)	(h)
$T_{>0.05 \mu M}$	36.7, 49.8, 53.2	26.0−41.7 (37.1)	(h)
Nadir	800, 1100, 1900	800−4100 (2000)	(/μl)
$T_{<2000}$	5.4, 5.9, 6.8	0−8.3 (0.5)	(days)
$A_{<2000}$	625, 4512, 5696	0−6731 (6) [c]	(days/μl)

a) Values for three patients.
b) Range (median).
c) $P=0.05$ vs. patients with fever.
AUC, area under the time versus concentration curve; $T_{>0.1 \mu M}$, time above concentration of 0.1 μM; $T_{>0.05 \mu M}$, time above concentration of 0.05 μM; Nadir, leukocyte nadir counts; $T_{<2000}$, time that leukocyte counts remain below 2000/μl: $A_{<2000}$, area between the curve of time versus leukocyte counts and the line of leukocyte counts of 2000/μl.

mated in all patients (Table II). Interpatient variability was large for A (CV=77%) and IC (59%) and moderate for T_{lag} (42%) and T_s (35%). When the correlation between pretreatment characteristics listed in Table I and pharmacodynamic parameters in Table II (A, IC, T_{lag}, and T_s) was evaluated, a negative correlation between age and IC, which was shown in the previous study,[15] was confirmed in this study ($r^2=0.21$, $P=0.07$, Fig. 5).

The pharmacodynamic model enabled us to estimate not only the lowest leukocyte count or surviving fraction, but also time-dependent variables, including the time of the nadir and the period of leukocyte counts below a certain value, e.g. 2000 or 4000/μl (Table III). Area between the curve of time versus leukocyte counts and the line of a certain value of leukocyte counts could also be estimated with the pharmacodynamic model (Fig. 1b). The measured nadir count (mean±SD=2100±900/μl, median 2000/μl) was not different from the nadir count predicted by the model (nadir count in Table III). The difference in the measured nadir count and the model-predicted nadir count in each patient was minimal (mean±SD=−35±300/μl,

median 0/μl). On the other hand, the time to the day of the observed nadir after the chemotherapy (mean±SD= 9.1±1.6 days, median 9 days) was significantly shorter than that to the nadir estimated by the model (nadir time in Table III, $P<0.001$). The difference of the measured versus model-predicted nadir time in each patient was 2.6±2.7 (median 2.2) days.

In a conventional pharmacodynamic analysis of paclitaxel, time above threshold concentration was reported to be a better predictor of leukopenia and neutropenia than AUC.[7, 8, 10] Although time above 0.1 μM ($r^2=0.16$) and 0.05 μM ($r^2=0.10$) showed a better correlation with the surviving fraction of leukocytes at the nadir than AUC ($r^2=0.02$) in this study, none of these correlations was significant.

Of the 17 patients, three developed fever during the leukopenic period. By comparing pharmacokinetic or pharmacodynamic parameters of these three patients to those of the remaining 14 patients without fever, parameters associated with fever were sought. As shown in Table IV, patients who did and did not experience fever had equiva-

lent AUC. Compared to patients without fever, patients with fever were exposed to concentrations of paclitaxel above 0.1 or 0.05 μM for a longer time, had lower leukocyte nadir counts, and had leukocyte counts below 2000/μl for a longer time. However, none of these differences was statistically significant. The only parameter which was significantly different for patients with and without fever was the area between the curve of time versus leukocyte counts and the line of the leukocyte count at 2000/μl (Table IV). There was no difference in the backgrounds of the patients as listed in Table I between those with and without fever.

Because the area between the curve of time versus leukocyte counts and the line of 2000/μl was the best predictor of fever, we sought to identify factors correlated to that area. The sensitivity parameter of the pharmacodynamic model, IC, was significantly correlated to the area ($r^2=0.27$, $P=0.04$), but pharmacokinetic parameters, including the AUC ($r^2=0.01$) and the time above a concentration of 0.1 μM ($r^2=0.04$) or 0.05 μM ($r^2=0.03$), did not show a correlation to the area.

DISCUSSION

The pharmacodynamic model used in this study adequately described the entire time course of leukopenia after treatment with paclitaxel infused over 3 h in patients treated in a phase II study against breast cancer. By predicting the time course of leukopenia, the model could estimate time-dependent aspects of leukopenia in each patient, including the time period during which leukocyte counts remained below 2000/μl and area between the curve of time versus leukocyte counts and the line of leukocyte values at 2000/μl. Fever associated with myelosuppression should depend on both the depth and the duration of leukopenia.[5] Therefore, the entire time course of leukopenia, not a singly measured nadir count, is clinically relevant. In this study, the area between the curve of time versus leukocyte counts and the line of the leukocyte count at 2000/μl was significantly associated with fever, but the association of the singly measured nadir count with fever was not significant. This observation supports the opinion that it is important to consider not only the degree of leukopenia, but also its time course in the pharmacodynamic analysis of anticancer agents. The model used in this study provided us with a novel instrument for the analysis of the time course of leukopenia.

Three of 17 patients developed fever in this study. Because of the small number of patients with fever, we could not definitively conclude that the area between the curve of time versus leukocyte counts and the line of the leukocyte count at 2000/μl was the best predictor of fever. The area between the curve of time versus leukocyte counts and the line of 1000/μl may be associated with

fever to a greater extent. However, the association of the area between the curve of time versus leukocyte counts and the line of leukocyte count at 1000/μl with fever could not be assessed in this study because only two patients had leukocyte counts less than 1000/μl. Neutropenia should be more closely associated with fever than leukopenia after chemotherapy. However, leukopenia was modeled in this study because the neutrophil counts were not measured at some points in a subset of patients. It was shown in the previous report that the pharmacodynamic model used in this study could be applied to the analysis of the time course of not only leukopenia, but also neutropenia if sufficient data on neutrophil counts were obtained.[15] The small number of patients in this study might preclude the generalization of the findings to the larger population of patients with breast cancer. By applying the model to larger numbers of different kinds of patient populations, the model may elucidate factors which are more relevant to neutropenic fever.

This study confirmed that the entire time course of leukopenia after treatment with paclitaxel infused over 3 h could be described by using the pharmacodynamic model. Estimated parameters of patients with breast cancer in this study were comparable to previously reported parameters of patients treated in a phase I study of paclitaxel with the same schedule.[15] The sensitivity parameter, IC, was 11.4±6.7 μg×h/ml for patients with breast cancer in this study and 12.1±6.1 μg×h/ml for 15 patients in the previous report. Likewise, the duration of sensitive stages of myeloid cells (T_s) was 219±76 vs. 288±64 h, relative compartment size of leukocytes in bone marrow (A) was 4.3±3.3 vs. 6.3±4.7, and the lag time before leukocyte counts began to decrease (T_{lag}) was 61±26 vs. 58±38 h. Physiologically, T_{lag} was analogous to transit time through the non-dividing maturation pool in bone marrow from metamyelocytes to segmented cells, which was reported in the literature to be 48 h in the presence of infection and 96 to 144 h under normal conditions.[22] These figures were close to the estimated T_{lag} in this study, 61±26 h. Similarly, T_s corresponded to transit time through mitotic compartments of myeloid cells (myeloblasts to myelocytes) which was reported to be 143 h.[24] The latter figure was within the range of estimated T_s in our patients (115 to 438 h). The estimated parameter of sensitivity (IC) was 11.4±6.7 μg×h/ml and close to the average AUC of paclitaxel in this study population, 23.8±7.1 μg×h/ml (Table II). This means that a change in paclitaxel AUC should result in a significant change in leukocyte production, because IC is the exposure causing 50% of the maximum inhibition of leukocyte production, which is at a steep portion of the E_{max} curve. The large interpatient variability of IC might be related, at least partially, to the variability of pharmacokinetic and pharmacodynamic processes including protein binding of the drug, as well as the

expression and function of P-glycoprotein in hematopoietic cells.

Leukopenia after chemotherapy should depend on various factors, including the sensitivity of bone marrow cells to the drug (corresponding to *IC* in the pharmacodynamic model), the size of the leukocyte pool in bone marrow (*A*), and the length of exposure to the drug (*T_s*). By separating each process, the pharmacodynamic model may elucidate the relationship between these processes and physiologic conditions. A significant negative correlation between age and the sensitivity parameter (*IC*) of the model was observed in the previous report on patients treated in a phase I study of paclitaxel.[15] In this study, the negative correlation was confirmed in a different set of patients treated in the phase II study of breast cancer. The exact reason why bone marrow cells in old patients show greater sensitivity to the same exposure to paclitaxel than those of young patients is not clear. Because factors stimulating leukocyte production should have been increased during the leukopenic period after chemotherapy, k_1 in the pharmacodynamic model should have been increased. However, the production of granulocytopoietic cytokines was reduced in the elderly,[25] and a decreased response to the granulocytopoietic stimuli in infection was reported in aged mice and humans.[26–28] It is plausible that the response to driving stimuli to leukocyte production during leukopenia was decreased in old patients with cancer. Accordingly, in a leukopenic period after chemotherapy, k_1 in the pharmacodynamic model should have been increased to a lesser extent in old patients than in young patients, but the model used in this study does not consider the change of the driving stimuli of leukocyte production and k_1 was fixed in both old and young patients to the same value (0.099/h). This may explain the negative correlation between age and the sensitivity parameter. Another factor which might be important in considering the reason for the correlation is protein binding. Unbound drug distributes to tissues and acts on receptors.[29] When the protein binding ratio is variable among patients, the free drug concentration is theoretically a better predictor of leukopenia of anticancer agents. If old patients had a higher fraction of unbound drug than young patients, this might explain the correlation between age and the sensitivity parameter. However, concentrations of protein-unbound drug were not measured in this study.

A full pharmacokinetic profile and the measurement of leukocyte counts more than once a week were necessary for fitting the pharmacodynamic model in each patient. However, a population approach with Bayesian estimation may be used to describe the full pharmacokinetic profile in each patient with a limited number of blood samplings.[30] Likewise, the number of leukocyte measurements might be reduced if the population approach could be used in conjunction with the pharmacodynamic model. By evaluating the time course of leukopenia in a large number of patients, the population approach might also contribute to the elucidation of the relationship between patient characteristics and parameters in the model and might lead to the prediction of the time course of leukopenia after chemotherapy with limited numbers of blood samplings and information on patient characteristics. Also a population approach can estimate the intra-patient variability of pharmacokinetic profiles and leukopenia, which was ignored in our model.

In conclusion, the pharmacodynamic model is a novel instrument for the analysis of the entire time course of leukopenia after anticancer agents. By describing the time course of leukopenia, the pharmacodynamic model revealed that fever in leukopenia was more closely associated with the time-dependent parameter of leukopenia, the area between the curve of time versus leukocyte counts and the line of leukocyte count of $2000/\mu l$, than the singly measured nadir count. The model was also helpful to elucidate the relationship between patient characteristics and factors contributing to leukopenia.

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