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ORIGINAL RESEARCH ARTICLE

An Algorithm and R Program for Fitting and Simulation of Pharmacokinetic and Pharmacodynamic Data

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Abstract

Background and Objectives Pharmacokinetic/pharmacodynamic link models are widely used in dose-finding studies. By applying such models, the results of initial pharmacokinetic/pharmacodynamic studies can be used to predict the potential therapeutic dose range. This knowledge can improve the design of later comparative large-scale clinical trials by reducing the number of participants and saving time and resources. However, the modeling process can be challenging, time consuming, and costly, even when using cutting-edge, powerful pharmacological software. Here, we provide a freely available R program for expediently analyzing pharmacokinetic/pharmacodynamic data, including data importation, parameter estimation, simulation, and model diagnostics.

Methods First, we explain the theory related to the establishment of the pharmacokinetic/pharmacodynamic link model. Subsequently, we present the algorithms used for parameter estimation and potential therapeutic dose computation. The implementation of the R program is illustrated by a clinical example. The software package is then validated by comparing the model parameters and the goodness-of-fit statistics generated by our R package with those generated by the widely used pharmacological software WinNonlin.

Results The pharmacokinetic and pharmacodynamic parameters as well as the potential recommended therapeutic dose can be acquired with the R package. The validation process shows that the parameters estimated using our package are satisfactory.

Conclusions The R program developed and presented here provides pharmacokinetic researchers with a simple and easy-to-access tool for pharmacokinetic/pharmacodynamic analysis on personal computers.

Key Points

The R program can handle pharmacokinetic/pharmacodynamic data analysis.

Sample running with real data demonstrates satisfactory results.

The R program is freely available to use.

1 Introduction

Dose-finding studies occupy a central place in the clinical development of new drugs [1]. They are crucial for defining the optimal dose range of a new drug, i.e., the clinical recommended dose for achieving the optimal therapeutic

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effect [2]. Conventionally, the effective therapeutic dose is explored in clinical trials, which are usually divided into phase I, II, and III trials [3, 4]. However, this approach requires considerable time, resources, and effort. Moreover, ethical issues are frequently encountered. Properly designed and accurately performed in early dose-finding studies can reduce the number of volunteers required in double-blind phase II trials and in comparative large-scale phase III trials, as well as reducing time and cost [2]. Pharmacokinetic/pharmacodynamic link models are widely used in dose-finding studies [5–7] and can facilitate the design of early clinical trials [8]. Such models link dose–concentration relationships (pharmacokinetics) with concentration–effect relationships (pharmacodynamics) to predict the time course of drug effects resulting from a certain dosage regimen [9]. Based on these relationship and known pharmacokinetic parameters, predictions of the intensity and decay of the pharmacological effect are possible.

Various software packages can be employed in the dose-selection process, e.g., WinNonlin, NONMEM, DAS, and 3P87/97. 3P87/97 can handle a variety of linear and nonlinear pharmacokinetic models, but it is unable to perform pharmacodynamic modeling. Consequently, it cannot establish the concentration–response relationship, making it less beneficial in the dose-selection process. DAS is commonly used in China, because of its broad pharmacological calculation functionality, including pharmacokinetics, pharmacodynamics, and the dynamics of drug interactions, among others. WinNonlin is a sophisticated industry-standard tool for nonlinear modeling that is particularly suited to non-compartmental analysis and pharmacokinetic/pharmacodynamic modeling. It facilitates simulations to evaluate data from bioavailability and clinical pharmacology studies. However, WinNonlin and DAS can carry out pharmacokinetic/pharmacodynamic analysis only for individual subjects: they cannot deal with the inter-individual variability. More precisely, they do not have the capacity to perform stochastic simulation, which is a key feature of many dose-selection decisions. NONMEM, a nonlinear mixed-effect modeling tool, remains the commonly used computational package for population-level pharmacokinetic/pharmacodynamic analysis. Several population analysis methods that can manage inter-individual and intra-individual variability are available in NONMEM, e.g., the iterative two-stage method, stochastic approximation, and Markov-chain Monte Carlo Bayesian analysis. However, because NONMEM is written in Fortran language, running the program is difficult for many new users [10]. In addition, their high price limits the utilities of these packages for some researchers. To summarize, as a consequence of limited features, operational difficulties, high prices, and low accessibility, these commercial software packages either cannot meet the needs of dose-finding studies or are not available to some researchers [11].

R software has gained popularity recently because of its powerful statistical techniques, support of object-oriented programming, and its accessibility for free of charge. The purpose of this study is to create a freely available R code package to support dose selection via a pharmacokinetic/pharmacodynamic link model. With this package, users enter experimental pharmacokinetic and pharmacodynamic data and, by changing a few user-specialized codes, can then quickly obtain the pharmacokinetic/pharmacodynamic parameters and a suggested effective therapeutic dosage. A clinical study is introduced to illustrate the operation of our R package. The package is validated by comparing parameters with those calculated using WinNonlin.

2 Materials and Methods

2.1 Phase I Study

The pharmacokinetic/pharmacodynamic data were obtained from a phase I clinical study on a new drug, pegylated recombinant human granulocyte colony-stimulating factor (PEG-G-CSF). It was a single-center, double-blind, dose-escalation, placebo-controlled study of single subcutaneous administration. The main inclusion criteria, exclusion criteria, ethical committee agreement, and all other important details can be found in Supplement 1. A total of 34 healthy subjects were randomly assigned to four different dosage groups: six healthy subjects each for the 30- and 200-μg/kg groups, and 11 healthy subjects each for the 60- and 100-μg/kg dosage groups. Each dosage group contained one placebo control who was excluded from the process of model fitting. Pharmacokinetic observations involved measuring the serum PEG-G-CSF concentration (ng/ml) immediately before administration and at 1, 3, 6, 8, 10, and 12 h and at 1, 2, 3, 4, 5, 6, 8, 10, 13, and 17 days after administration. Pharmacodynamic observations involved measuring the value of absolute neutrophil count (ANC, 10^9/l) before administration and at 3, 6, 8, 10, and 12 h and at 1, 2, 3, 4, 5, 6, 7, 9, 11, 14, 18, and 22 days after administration.

2.2 Data Analysis

We write a software package in R (Version 3.2.3, The R Foundation for Statistical Computing, Vienna, Austria) for pharmacokinetic/pharmacodynamic analysis. We use linear regression and linear least-squares methods for parameter estimation. To manage inter-individual variability (by

modifying the model parameters), we adopt the standard two-stage (STS) approach. The potential recommended therapeutic dose can then be obtained using the sigmoid $E_{\max}$ model. Finally, our software package is validated by comparing the model parameters obtained using our software and Phoenix WinNonlin (Build 6.1.0.173, Pharsight Corporation, Mountain View, CA, USA). The main steps of our algorithm are explained in the following sections, and the details are given in Supplement 2.

2.2.1 Parameter Estimation

The STS approach performs pharmacokinetic/pharmacodynamic analysis in two steps [13]. The first step involves fitting a model to individual data and estimation of the individual parameters, with no predefined relationship between different individuals in the population. These individual parameters are then used in the second step, in which the average parameters (mean) and variability (standard deviation, SD) in the population are calculated.

2.2.1.1 The First Step: Individual Data Fitting A pharmacokinetic/pharmacodynamic link model is needed to analyze the relationship between drug concentration and effect for individual data. The model is formalized in the following equations:

$$C = f(k;t) \tag{1}$$

$$C_e = f(k, k_e; t) \tag{2}$$

$$E = E_0 + \frac{E_m C_e^{\gamma}}{EC_{50}^{\gamma} + C_e^{\gamma}} \tag{3}$$

where C and C_e are the serum concentration in the central compartment and the effect compartment, and E is the effect of the drug. E_0 and E_m are, respectively, the initial effect and the difference between the maximal effect and the initial effect, whereas EC_{50} is the concentration at 50 % maximal effect. γ is the binding coefficient and k is the vector of transfer rates, incorporating k_{01}, the transfer rate of drug from the site of absorption to the central compartment; k_{10}, the elimination rate from the central compartment; and k_{12} and k_{21}, the transfer rates between the central compartment and the peripheral compartment. k_e is the transfer rate from the central compartment to the effect compartment (or biophase).

We chose a compartment model for pharmacokinetic analysis and the sigmoid $E_{\max}$ model for the pharmacodynamic analysis, because of their universality in the pharmacokinetic/pharmacodynamic link model [9]. We introduced an effect compartment to deal with the time lag between C and E [13, 14]. In other words, the relationship between C and E can be replaced by the relationship between C_e and E. The latter relationship can be directly processed by the sigmoid $E_{\max}$ model, and the pharmacokinetic and pharmacodynamic models are thereby linked [15–18].

The two-compartment model is used to illustrate the algorithm in detail without loss of generality. The model is expressed as follows:

$$C = Le^{-\alpha t} + Me^{-\beta t} + Ne^{-k_{10}t} \tag{1-1}$$

$$C_e = k_e M e^{-k_e t}\left(\frac{e^{(k_e-\alpha)t}-1}{k_e - \alpha} + \frac{e^{(k_e-\beta)t}-1}{k_e - \beta} + \frac{e^{(k_e-\mathrm{k}_{01})t}-1}{k_e - k_{01}}\right) \tag{2-1}$$

$$E = E_0 + \frac{E_m C_e^{\gamma}}{EC_{50}^{\gamma} + C_e^{\gamma}} \tag{3-1}$$

Assume that the sample data are denoted as E and the respective observation time is denoted as T. After a value of k_e is given, which is denoted as k_{e0}, the pattern of the sigmoid $E_{\max}$ model can be rewritten as:

$$\frac{E_m}{E - E_0} - 1 = \frac{EC_{50}^{\gamma}}{EC_{50}^{\gamma} + C_e^{\gamma}} \tag{3-2}$$

The following equation for $C_{(e0,T)}$, the concentration of effect compartment in a certain time, shows that the value of C is affected by k_{e0} and T:

$$C_{(e0,T)} = k_{e0} M e^{-k_{e0}T}\left(\frac{e^{(k_{e0}-\alpha)T}-1}{k_{e0} - \alpha} + \frac{e^{(k_{e0}-\beta)T}-1}{k_{e0} - \beta} + \frac{e^{(k_{e0}-\mathrm{k}_{01})T}-1}{k_{e0} - k_{01}}\right) \tag{2-2}$$

This equation is derived by integrating differential equations with the initial condition $C_e(0) = 0$. The left side of Eq. (3–2) is always greater than 0 for the assumption $E_0 = 0.95E_{\min}$ and $E_m = 1.05E_{\max}$. By taking the logarithm of both sides of the equation and denoting the left side as E_1, then $\ln(E_1) = -\gamma \ln C_{(e0,T)} + \gamma \ln \mathrm{EC}_{50}$ (3–3). The parameters of the above regression can be obtained by the linear least squares method. Denoting the regression vector $\begin{pmatrix} -\gamma \\ \gamma \ln \mathrm{EC}_{50} \end{pmatrix} = \begin{pmatrix} a \\ b \end{pmatrix}$, then

$$\mathrm{EC}_{50} = e^{-\frac{b}{a}} \tag{4}$$

For k_{e0},

$$E = \frac{E_m}{e^{a \ln C_{(e0,T)}} + 1} + E_0 \tag{5}$$

Because k_e and k are usually the same order of magnitude, the interval for k_e can be estimated as $0.5k < k_e < 10\ k$. For a step length of, say, 0.01, all the values close to k_e can be tried. For instance, when $k_e = 0.5\ k$, group $(k_e, \mathrm{EC}_{50}, \gamma)_1$ can be generated; when

$k_e = 0.5k + 0.01$, group (k_e, EC_{50}, γ)$_2$ can be generated, and so on. This procedure can be carried out easily on a personal computer. The value of k_e that minimizes the sum of the squares of the error (SSE) of the effect is k_{e0}, and the acceptable values of parameters are (k_{e0}, EC_{50}, γ). *SSE* is given by:

$$\mathrm{SSE} = \sum \left(E - \hat{E}\right)^2 \tag{6}$$

where E is observed effect and $\hat{E}$ is fitted effect.

2.2.1.2 The Second Step: Population Analysis Population characteristics $\hat{\beta}_{\mathrm{STS}}$ and $\hat{D}_{\mathrm{STS}}$ of each parameter are estimated as the empirical mean (arithmetic or geometric) and variance of the individual parameters according to the following equations:

$$\hat{\beta}_{\mathrm{STS}} = \frac{1}{N}\sum_{j=1}^{N} \hat{\beta}_j \tag{7}$$

$$\hat{D}_{\mathrm{STS}} = \frac{1}{N}\sum_{j=1}^{N} \left(\hat{\beta}_j - \hat{\beta}_{\mathrm{STS}}\right)^2 \tag{8}$$

where $\hat{\beta}_j$ is the estimate of individual parameter. The standard deviation ($\hat{S}_{\mathrm{STS}}$) is estimated as the square root of $\hat{D}_{\mathrm{STS}}$. $N - 1$ can be used instead of N in the denominator of the variance estimate.

2.2.2 Computation of Appropriate Dosage

According to population pharmacokinetic/pharmacodynamic estimates, several groups of dosages and their corresponding effects can be produced. The relationship between them can be analyzed using the sigmoid $E_{\max}$ model, whereas the parameters can be computed by linear transformation, as mentioned in Sect. 2.2.1. Accordingly, the dosage corresponding to a certain percentage of the maximum effect is taken as the appropriate dosage. The steps below show the algorithm for computing the potential recommended therapeutic dosage.

2.2.2.1 The First Step: Computing the Average Serum Concentration The average serum concentration (C_{av}), which is the average concentration of a drug between absorption and excretion, can be computed as follows:

$$C_{\mathrm{av}} = \frac{\mathrm{AUC}}{t} \tag{9}$$

where AUC is the area under the concentration–time curve. AUC can be calculated from the pharmacokinetic model by integrating the concentration over time. t is the clinically effective duration, which can be determined on the basis of clinical experience, and is normally the time when the drug concentration approaches zero.

2.2.2.2 The Second Step: Computing the Average Effect The effect correlated to C_{av} is E_{av}. With all the parameters of the pharmacokinetic/pharmacodynamic link model have been generated, the effect can be computed once the concentration is given. Because the pharmacokinetic model gives the relationship between concentration and time, the t value that produces the smallest difference between C and C_{av} can be considered as the equivalence time t_{av}. Then, an equivalence concentration of the effect compartment, Ce_{av}, can be computed as:

$$\mathrm{Ce}_{\mathrm{av}} = k_{e0}e^{-k_{e0}t_{\mathrm{av}}}\left(\frac{L}{k_{e0} - \alpha}\left(e^{(k_{e0}-\alpha)t_{av}} - 1\right) + \frac{M}{k_{e0} - \beta}\left(e^{(k_{e0}-\beta)t_{\mathrm{av}}} - 1\right) + \frac{N}{k_{e0} - k_{10}}\left(e^{(k_{e0}-\mathrm{k}_{01})t_{\mathrm{av}}} - 1\right)\right) \tag{2-3}$$

where L, M, N, α, β, k_{10}, and k_{e0} are given by the pharmacokinetic model. Finally, E_{av} can be computed as:

$$E_{\mathrm{av}} = E_0 + \frac{E_m \mathrm{Ce}_{\mathrm{av}}^{\gamma}}{\mathrm{EC}_{50}^{\gamma} + \mathrm{Ce}_{\mathrm{av}}^{\gamma}} \tag{3-3}$$

where E_0 and E_m, respectively, are the initial value of the effect and the range of the effect correlated to a certain dosage. EC_{50} and γ are given by the pharmacodynamic model.

2.2.2.3 The Third Step: Computing the Relationship Between C_{av} and E_{av} The selection of a dosage is based on C_{av}s and E_{av}s, where s indicates different dosage groups. Each dosage D_i ($i = 1, 2, 3\ldots$) can be fitted by the sigmoid $E_{\max}$ model. The parameters of the sigmoid $E_{\max}$ model of C_{av} and E_{av} can be found in the same way, as data are fitted using the pharmacodynamic model. EC_{50} is given as one of the parameters of the model, and then EC_{90} (the concentration when the effect reaches 90 %) is given by:

$$\mathrm{EC}_{90} = e^{\log \mathrm{EC}_{50} + \log\left(\frac{E-E_0}{[E_m-(E-E_0)]^{\gamma}}\right)} \tag{10}$$

where E_0 and E_m are the initial value and range, respectively, of E_{av}s. In most cases, E_0 is zero. The relationship between a given dose and the average concentration can then be established. The appropriate dosage D is usually set as the dose whose corresponding average concentration is close to EC_{90}. In addition, researchers can determine the potential recommended dose by combining the model fitting results and clinical practice.

3 Results

The R code package written to perform the pharmacokinetic/pharmacodynamic analysis outlined in the above algorithm is given in the "Appendix". The package

Table 1 Fitting results of each dosage of pegylated recombinant human granulocyte colony-stimulating factor by the pharmacokinetic model

Time (h)	30 μg/kg			60 μg/kg			100 μg/kg			200 μg/kg		
	C-obs[a]	C-pred[b]	Error (%)[c]	C-obs	C-pred	Error (%)	C-obs	C-pred	Error (%)	C-obs	C-pred	Error (%)
1	8.308	10.675	28.49	14.876	16.079	8.09	20.535	27.909	35.91	46.844	75.279	60.70
3	23.554	41.650	76.83	50.796	62.503	23.05	82.500	138.234	67.56	166.994	287.489	72.16
6	29.088	46.445	59.67	78.543	89.378	13.79	168.847	228.862	35.54	316.582	492.962	55.71
8	36.014	40.482	12.41	94.268	92.926	−1.42	190.294	256.096	34.58	391.094	574.124	46.80
10	34.006	33.241	−2.25	98.842	91.011	−7.92	228.443	266.170	16.51	442.450	622.621	40.72
12	29.466	26.553	−9.89	99.519	86.340	−13.24	228.477	264.672	15.84	482.786	646.352	33.88
24	10.400	7.641	−26.53	60.219	51.474	−14.52	211.841	172.867	−18.40	506.110	538.340	6.37
48	4.790	3.446	−28.06	13.071	17.913	37.04	64.231	45.902	−28.54	332.254	202.130	−39.16
72	3.544	2.541	−28.29	4.516	7.845	73.72	8.613	13.931	61.74	89.686	67.049	−25.24
96	1.764	1.900	7.73	3.604	4.338	20.38	4.733	6.225	31.52	13.160	24.835	88.72
120	1.252	1.421	13.52	2.555	2.791	9.22	3.336	3.778	13.26	7.718	11.430	48.10
144	0.736	1.063	44.43	1.601	1.930	20.52	1.902	2.616	37.54	4.596	6.518	41.82
192	0.436	0.595	36.38	0.659	0.989	50.08	0.987	1.378	39.63	2.738	2.971	8.52
240	0.216	0.333	54.00	0.455	0.518	13.75	0.571	0.740	29.54	1.266	1.541	21.72
312	0.100	0.139	39.17	0.171	0.197	15.08	0.297	0.291	−1.86	0.538	0.593	10.29
408	0.072	0.044	−39.51	0.096	0.054	−43.51	0.114	0.084	−26.13	0.230	0.167	−27.42

[a] The original observed mean serum concentration (C-obs) of individuals (ng/ml) in each dosage group

[b] The predicted mean serum concentration (C-pred) by pharmacokinetic model (ng/ml) in each dosage group

[c] The relative error between C-obs and C-pred

contains four parts: data processing, parameter estimation, simulation, and model diagnostics. The results of each step as applied to the data generated in a clinical study are presented below.

Step 1 Data Processing

All the relevant data generated by the clinical trial are put in a csv file, with observations recorded by columns giving patient ID, time, drug concentration, and the effect (lines 1–49).

Step 2 Parameter Estimation

The algorithm for estimating the pharmacokinetic parameters k_{01}, k_{10}, k_{12}, and k_{21}; the pharmacodynamic parameters k_{e0}, γ, and EC_{50}; and the related C_{av} and E_{av} values for each individual in each dosage group were coded using the linear least-squares method in lines 50–153.

The Akaike information criterion (AIC) is applied to select whether the one-compartment model or the two-compartment model should be used. Consequently, the two-compartment model is chosen for the pharmacokinetic analysis, and the target time is set at 120 h, i.e., AUC_{0-120} is computed in our example. This time was selected, because the serum concentration of the drug is very small after 120 h. Consequently, 120 h was considered to be the clinically effective period for the drug PEG-G-CSF.

Step 3 Simulation

Simulation of the clinical study data was performed in lines 154–326. The results fall into four categories: fitted concentration–time curves for each individual and comparison with the original data (Table 1; Fig. 1); fitted effect–time curves for each individual and a comparison with the original data (Table 2; Fig. 2); the relationship between C_{av} and E_{av} determined by the sigma E_{max} model based on four dosages (Table 3; Fig. 3); and a linear relationship between the dose and AUC_{0-120} (Fig. 4). The final parameters are calculated by the population pharmacokinetic STS method, which produces mean value and SD of individual parameters in each dosage group (Table 4).

Note that the initial concentration is zero in the study. Because G-CSF is normally present in the human body at very low concentrations, we regard the pre-administration level as the background value. The observed concentrations (Table 1) have had this background value subtracted. The observed concentration/effect data presented in Tables 1 and 2 are mean values of the individual concentrations/effects in each dosage group. The predicted concentration/effect data were fitted with the STS method rather than simply using the mean concentration/effect to fit the model.

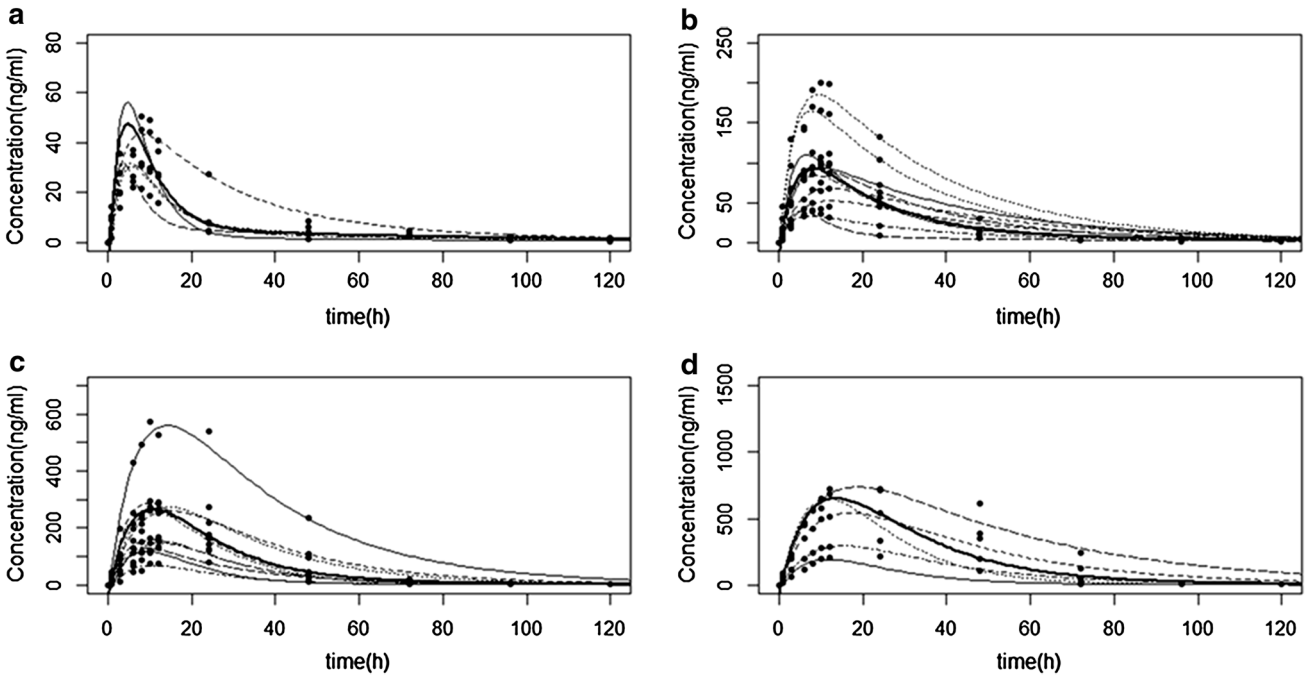

Fig. 1 Fitted results of our pharmacokinetic model for dosages of PEG-G-CSF 30, 60, 100, and 200 μg/kg (**a**–**d**, respectively). The data points show the observed serum PEG-G-CSF concentrations for each individual. The *dashed lines* show the fitted curves for each individual. The *solid line* is the fitted concentration–time relationship of the population derived using the STS method. *PEG-G-CSF* pegylated recombinant human granulocyte colony-stimulating factor

Step 4 Model Diagnostics

The statistical criteria for evaluating the goodness-of-fit of the model, including the coefficient of determination (R^2), the AIC, and Bayesian information criterion (BIC), are calculated in lines 327–380. The results are presented in Table 4.

3.1 Estimation of the Potential Recommended Therapeutic Dosage

The potential recommended therapeutic dosage is usually taken as the dosage that can achieve a given percentage of E_{max} (EC_{90} in this case). As shown in Fig. 4, there is a linear relationship between AUC_{0-120} (or C_{av}) and dose (D). We obtain $EC_{90} = 24.49$ (ng/ml) by the sigmoid E_{max} model. Considering individual diversity and optimizing the efficacy of the drug, we could select 60 μg/kg (which achieved 88.89 % of E_{max}) as the potential recommended therapeutic dose on a weight basis, and 3.6 mg for a standard weight of 60 kg.

4 Discussion

4.1 Applicability of Our Pharmacokinetic/Pharmacodynamic Model

To set up a pharmacokinetic/pharmacodynamic link model to carry out dose exploration, it is necessary to select appropriate pharmacokinetic and pharmacodynamic models. Compartment models are widely used in pharmacokinetic analysis [19, 20], and we use AIC criteria to determine the selection of a one-compartment or a two-compartment model. We employ the two-compartment extravascular model for the pharmacokinetic analysis, because PEG-G-CSF is administered by subcutaneous injection, and its pharmacokinetics is compatible with the two-compartment model. Consequently, our software package is applicable only to extravascular administration and is not appropriate for intravascular administration. There are many other commonly used modeling methods, such as the non-compartmental model, that could have been used to calculate the pharmacokinetic parameters without assuming the number of compartments [21]. However, in the analysis of the pharmacokinetic/pharmacodynamic model, it is necessary to use a compartment model for pharmacokinetic analysis. As a preliminary exploration of a pharmacokinetic/pharmacodynamic link model coded in R, we select the classic compartment method without loss of generality.

With concentration and response data on the effect compartment, Several basic pharmacodynamic models, such as the fixed effect model, the linear model, the log–linear model, the E_{max} model, and the sigmoid E_{max} model, can be used to extract pharmacodynamic parameters from the concentration and response data [22]. As a generalization of the E_{max} model and an empirical function for

Table 2 Fitting results of each dosage of pegylated recombinant human granulocyte colony-stimulating factor by the pharmacodynamic model

Time (h)	30 μg/kg			60 μg/kg			100 μg/kg			200 μg/kg		
	E-obs[a]	E-pred[b]	Error (%)[c]	E-obs	E-pred	Error (%)	E-obs	E-pred	Error (%)	E-obs	E-pred	Error (%)
0	3.222	1.786	−44.57	2.902	1.787	−38.43	3.053	1.520	−50.21	2.526	1.539	−39.07
3	2.064	2.464	19.36	2.261	1.872	−17.18	1.826	1.786	−2.21	2.698	1.645	−39.01
6	6.326	6.535	3.31	5.355	2.189	−59.12	6.061	5.278	−12.91	5.070	3.177	−37.33
8	15.526	10.013	−35.51	13.434	3.047	−77.32	14.068	10.239	−27.22	12.488	6.821	−45.38
10	16.760	15.886	−5.22	16.860	4.843	−71.27	16.108	15.210	−5.57	15.230	10.449	−31.39
12	20.404	19.474	−4.56	17.435	7.817	−55.16	20.152	18.695	−7.23	18.646	13.989	−24.98
24	18.116	24.044	32.72	19.157	21.337	11.38	22.360	27.493	22.96	18.840	24.766	31.46
48	22.436	23.599	5.18	22.369	25.757	15.14	27.117	28.453	4.93	24.648	27.727	12.49
72	18.932	22.025	16.34	27.120	24.682	−8.99	25.041	27.326	9.12	28.398	27.158	−4.37
96	19.314	19.872	2.89	22.818	22.005	−3.56	27.651	24.301	−12.12	22.400	25.437	13.56
120	16.274	17.049	4.76	12.593	17.429	38.40	17.888	20.575	15.02	24.964	22.713	−9.02
144	11.138	14.114	26.72	8.659	11.687	34.97	13.805	16.682	20.84	16.222	19.159	18.11
168	11.254	11.308	0.48	10.006	7.439	−25.65	13.580	12.543	−7.64	13.188	15.183	15.13
216	10.772	6.694	−37.86	7.400	2.912	−60.65	8.860	6.295	−28.95	12.970	6.473	−50.09
264	8.768	3.766	−57.04	5.121	2.067	−59.64	6.443	3.471	−46.12	7.774	2.625	−66.24
336	4.130	2.044	−50.51	4.224	1.861	−55.95	4.643	2.004	−56.84	4.888	1.682	−65.60
432	3.618	1.787	−50.61	3.092	1.801	−41.75	2.887	1.570	−45.62	3.850	1.603	−58.37
528	3.420	1.786	−47.78	2.602	1.788	−31.30	3.010	1.520	−49.50	2.688	1.546	−42.48

[a] The original observed mean effect (E-obs) of individuals (10^9/l) in each dosage group

[b] The predicted mean effect (E-pred) by pharmacodynamics model (10^9/l) in each dosage group

[c] The relative error between E-obs and E-pred

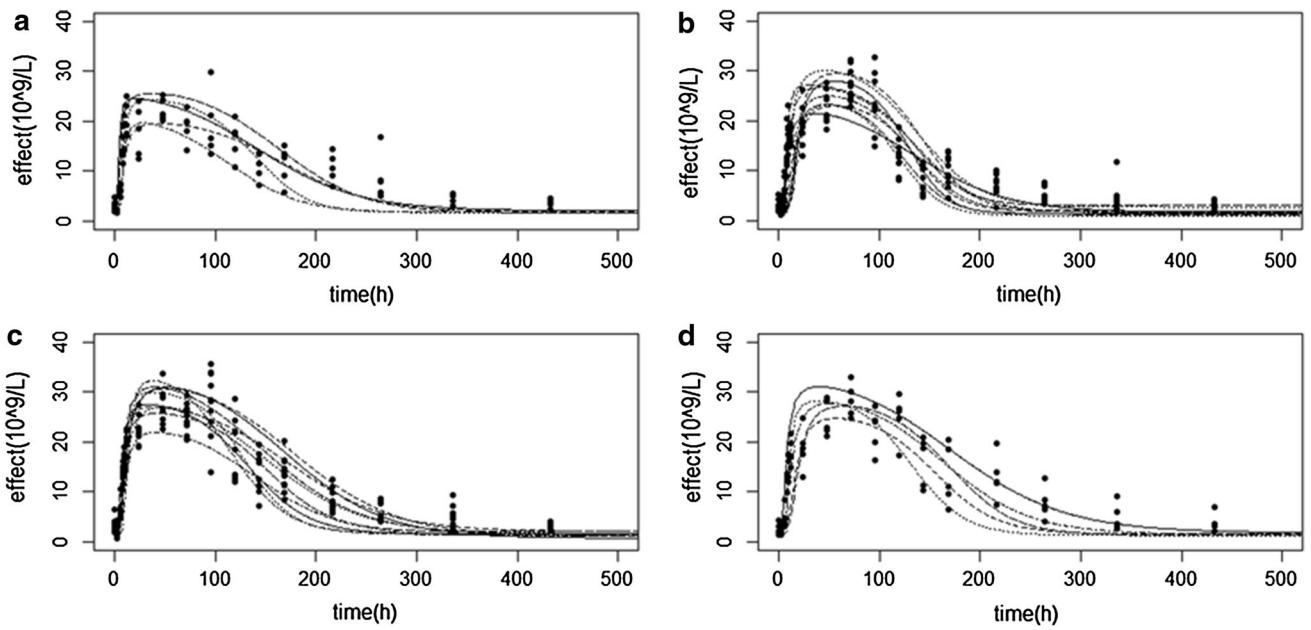

Fig. 2 Fitted results of our pharmacodynamic model for dosages of pegylated recombinant human granulocyte colony-stimulating factor 30, 60, 100, and 200 μg/kg (**a**–**d**, respectively). The data points show the observed effects (i.e., the absolute neutrophil counts, ANC) for each individual. The *dashed lines* show the fitted effect– time relationship for each individual. The *solid line* is the fitted effect–time relationship of the population derived using the STS method

Table 3 C_{av} and E_{av} of each dosage of pegylated recombinant human granulocyte colony-stimulating factor by the pharmacokinetic–pharmacodynamic model

D^a (μg/kg)	C_{av}^b (ng/ml)	E_{av}^c (10^9/l)
30	8.17 (3.60)	22.60 (2.74)
60	32.35 (17.48)	25.67 (2.96)
100	72.27 (58.12)	28.41 (3.14)
200	200.14 (137.77)	27.76 (2.29)

In brackets is standard deviation

[a] The dosage of administration

[b] The average concentration (ng/ml) of each dosage from 0 to 120 h by pharmacokinetic model

[c] The effect (ANC, 10^9/l) corresponding to each C_{av} by the pharmacokinetic–pharmacodynamic model

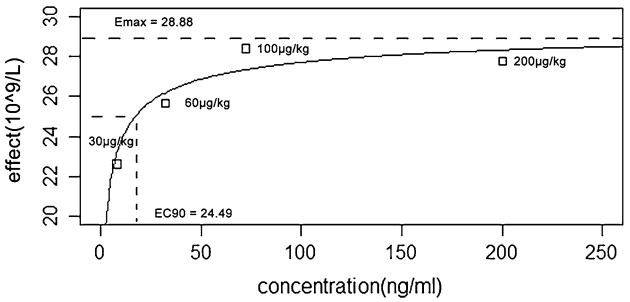

Fig. 3 Results of the average concentration (C_{av}) and average effect (E_{av}) determined by our pharmacokinetic/pharmacodynamic link model. The fitted curve shows the relationship between the concentration and effect based on the sigmoid E_{max} model

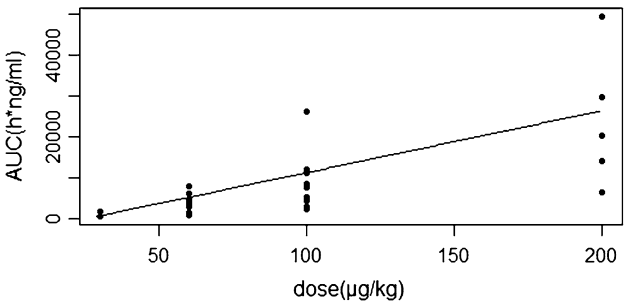

Fig. 4 Relationship between AUC_{0-120} and dosage. The *dots* represent the AUC_{0-120} of each individual in each dosage group. The *line* shows the fitted relationship between AUC_{0-120} and dosage

describing the nonlinear concentration relationship, the sigmoid E_{max} model is classical and is typically used [23–27]. With the compartment model and the sigmoid E_{max} model, the effect can be computed once the concentration is given [28]. Note that this characterization of the dose–concentration–effect relationship is essentially confined to drugs that have a direct correlation between the measured concentration and the observed effect [9]. It is not appropriate for drugs for which the peaks of concentration and effect are not reached concurrently. Consequently, we introduce an effect compartment to deal with the time lag between concentration and effect [13, 14].

To summarize, we adopt the commonly used and widely applicable compartment model and sigmoid E_{max} model to establish a pharmacokinetic/pharmacodynamic link model. In future work, we will consider introducing alternative pharmacokinetic models, such as the non-compartment model, and alternative pharmacodynamic models, such as the mixed-effect model, to improve the flexibility and applicability of our package for pharmacokinetic/pharmacodynamic analysis.

4.2 Validation of Our Pharmacokinetic/Pharmacodynamic Analysis

There are several other pharmacological software packages that can perform pharmacokinetic/pharmacodynamic analysis. To validate our package, we compare our model parameters and model diagnostic statistics with those calculated by the widely used software WinNonlin. The pharmacokinetic model both in our package and in WinNonlin is a two-compartment model, and both use the sigmoid E_{max} model for the pharmacodynamic analysis. Our algorithm is the linear least-squares approach, whereas the algorithm in WinNonlin is the Gauss–Newton method with the Levenberg and Hartley modification. A comparison of the two software packages is given in Table 4. For the four dosage groups, 20 of the 32 parameters calculated using our package are within 30 % difference of the values calculated using WinNonlin. The R^2 values for the goodness-of-fit of our model are all greater than 0.7. Moreover, the differences in R^2, AIC, and BIC between the two packages are all less than 40 %. Based on this comparison, we conclude that our package's performance is satisfactory.

4.3 Limitations of Our Algorithm

Certainly, there are large differences for some parameters in Table 4. For example, the difference for E_0 in the 60 μg/kg group is greater than 55 %, and the difference for k_{21} in the 100 μg/kg group is greater than 46 %. Moreover, all the goodness-of-fit statistics for the WinNonlin model are smaller than those for our model, which indicates that WinNonlin does a better job of model fitting. These differences result from the different algorithms used for model fitting. Our package uses linear least-squares method to estimate parameters. In our study, neither the compartment model nor the sigmoid E_{max} model is fundamentally linear. We use a log algorithm to change them into a linear form to make them compatible with linear regression

Table 4 Detailed comparison of *R* estimates with those of WinNonlin by pegylated recombinant human granulocyte colony-stimulating factor dosage

Parameter	Unit	30 μg/kg		60 μg/kg		100 μg/kg		200 μg/kg	
		R	WinNonlin	*R*	WinNonlin	*R*	WinNonlin	*R*	WinNonlin
Pharmacokinetics									
Parameters									
k_{01}^{a}	h^{-1}	0.166 (0.022)	0.155 (0.022)	0.136 (0.030)	0.125 (0.063)	0.072 (0.021)	0.095 (0.053)	0.052 (0.003)	0.055 (0.007)
k_{10}^{b}	h^{-1}	0.092 (0.045)	0.087 (0.030)	0.038 (0.019)	0.077 (0.017)	0.058 (0.019)	0.067 (0.010)	0.049 (0.025)	0.055 (0.006)
k_{12}^{c}	h^{-1}	0.072 (0.064)	0.053 (0.031)	0.014 (0.029)	0.019 (0.015)	0.008 (0.008)	0.002 (0.003)	0.005 (0.004)	0.0004 (0.0003)
k_{21}^{d}	h^{-1}	0.023 (0.009)	0.017 (0.009)	0.018 (0.004)	0.013 (0.011)	0.015 (0.003)	0.007 (0.006)	0.015 (0.002)	0.007 (0.006)
Diagnostics									
R^2		0.917 (0.052)	0.982 (0.120)	0.958 (0.023)	0.989 (0.012)	0.961 (0.031)	0.977 (0.030)	0.949 (0.038)	0.987 (0.006)
AIC		102.914 (14.156)	75.367 (18.468)	128.499 (17.791)	95.810 (19.353)	155.341 (18.104)	134.440 (19.119)	187.494 (18.216)	159.721 (20.974)
BIC		107.913 (14.156)	80.367 (18.468)	133.499 (17.791)	100.809 (19.353)	160.340 (18.104)	139.439 (19.119)	192.494 (18.216)	164.720 (20.974)
Pharmacodynamics									
Parameters									
E_0^{e}	10^9/l	1.845 (0.156)	3.009 (0.700)	1.890 (0.625)	3.415 (0.867)	1.522 (0.429)	2.987 (1.108)	1.541 (0.200)	2.681 (2.207)
EC_{50}^{f}	10^9/l	2.911 (0.908)	3.097 (1.493)	15.175 (9.462)	12.632 (11.036)	22.560 (17.187)	50.475 (83.702)	71.729 (53.634)	70.331 (39.751)
E_{max}^{g}	10^9/l	25.859 (3.694)	23.632 (8.624)	28.973 (3.155)	21.789 (3.248)	31.838 (4.153)	31.680 (11.073)	31.013 (2.429)	29.238 (9.151)
Gamma[h]		2.626 (0.770)	2.493 (1.257)	3.472 (0.923)	2.915 (1.925)	2.101 (0.789)	1.525 (0.628)	2.169 (0.524)	1.892 (1.707)
Diagnostics									
R^{2i}		0.827 (0.080)	0.875 (0.068)	0.721 (0.109)	0.839 (0.200)	0.887 (0.055)	0.923 (0.026)	0.768 (0.072)	0.894 (0.022)
AIC[j]		101.032 (11.830)	91.546 (11.789)	113.089 (9.478)	96.603 (14.055)	100.250 (9.474)	93.491 (8.182)	114.864 (4.775)	99.704 (5.107)
BIC[k]		103.704 (11.830)	95.941 (11.758)	115.760 (9.478)	101.054 (14.055)	102.921 (9.474)	97.942 (8.182)	117.535 (4.775)	104.155 (5.107)

All the parameters are the mean value of individual parameter in each dosage group. In brackets is standard deviation

[a] The transfer rate from where the drug is absorbed and central compartment of individuals

[b] The elimination rate from central compartment to peripheral compartment

[c] The transfer rate from central compartment to peripheral compartment

[d] The transfer rate from peripheral compartment to central compartment

[e] The initial effect

[f] The concentration when 50 % maximal effect reaches

[g] The maximal effect

[h] The binding coefficient

[i] The coefficient of determination

[j] Akaike's information criterion

[k] The Bayesian information criterion

methods. Consequently, errors occur in the estimation of both pharmacokinetic and pharmacodynamic parameters. Furthermore, because the linear least-squares method is applied twice in the two-compartment model, the second step (based on the first step) enlarges the error. Nonlinear regression has some advantages compared with linear regression [29–31]. If the process is inherently nonlinear, nonlinear regression (such as the Gauss–Newton method employed in WinNonlin) can describe it better than linear regression. We adopt linear least-squares regression for the preliminary exploration of pharmacokinetic/pharmacodynamic analysis, because of its simplicity and effectiveness. In future work, we may consider nonlinear methods to improve parameter estimation.

Another source of error is that the pharmacokinetics of biological products, such as PEG-G-CSF, exhibit relatively large inter-individual variability [32, 33]. The observed mean concentration/effect for individuals in each group presented in Tables 1 and 2 actually have large standard variation. Therefore, as we mentioned before, we adopt a typical population pharmacokinetic method, STS method rather than directly using the mean concentration/effect data, to estimate the model parameters and predict the concentration/effect. Therefore, despite fitting the population data well, our model would generate large errors (Tables 1, 2), because of the large variability of the observed data. The STS method is a simple approach for pooling individual estimates of the model parameters. However, simply calculating the mean and standard deviation of individual parameters tends to overestimate parameter dispersion. Moreover, if the modeling is performed with sparse data, the STS method may run into practically unidentifiable parameters or problems resulting from the model selection [34, 35]. Several advanced population pharmacokinetic methods can handle these inter-individual and intra-individual variabilities. These methods include the global two-stage method, the iterative two-stage method, the Bayesian two-stage method, the nonlinear mixed effects model, and nonparametric methods [36, 37]. We may consider incorporating some of these techniques into our package to improve the handling of inter-individual variability in the future.

4.4 Meaning for Drug Research

To ensure efficacy and safety, a key objective in new drug research is to estimate an effective therapeutic dose. If the dose is too low, treatment may prove ineffective. If the dose is too high, problems of safety and tolerability may arise [38]. Using pharmacokinetic/pharmacodynamic modeling in phase I clinical trials involving dose exploration can save a lot of time and resources. We aim to implement the complex pharmacokinetic/pharmacodynamic data fitting process by producing a software package that is compact, flexible, readily available, and can be widely used. R is fully free computational programming software that is becoming increasingly popular worldwide. Users of our package can import their pharmacokinetic/pharmacodynamic data and quickly obtain parameter estimates and the dose-exploration range.

5 Conclusions

Dose-finding studies are of great importance in clinical pharmacology, especially those studies dedicated to finding the dose that can achieve a certain percentage of the maximal treatment effect. Many current commercially available pharmacological analysis packages require a steep learning curve. We have shown that it is possible to model pharmacokinetic/pharmacodynamic data and address optimal dose problems in a package based on the R programming language. We present our algorithm and demonstrate the accessibility and feasibility of the package by modeling data obtained in a clinical study. The validation procedure implemented by the widely accepted software WinNonlin illustrates that our package is satisfactory. Although the package has limitations in respect of the types of pharmacokinetic/pharmacodynamic model and model fitting algorithm employed, we have created a simple, freely available tool to fit pharmacokinetic/pharmacodynamic data that can be used by those who do not have access to commercial pharmacological software. The next step in our research will address the limitations and make the R package more applicable.

Compliance with Ethical Standards

Conflict of interest Jijie Li, Kewei Yan, Lisha Hou, Xudong Du, Ping Zhu, Li Zheng, and Cairong Zhu declare that they have no conflict of interest.

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Appendix

```
---
title: "An Algorithm and R Program for Fitting and Simulation of PK/PD Data"
author: "Jijie Li, Kewei Yan, Lisha Hou,Xudong Du, Ping Zhu, Li Zheng, Cairong Zhu"
date: "2016.7.18"
output: word_document
---

```{r, message=FALSE, results="asis",echo=FALSE}
# ============================
# This code contains 4 parts:
# data process
# pkpd parameter estimation
# simulation
# model diagnostics
# ======================
# =part 1 data process=
# ======================
# observations are recorded by columns, marked with different patient ID and time, since observations of pk and pd are not recorded by exactly the same time, two data files are needed
pkdata0 <- read.csv(file = "D:/pkdata.csv", head = TRUE)
pddata0 <- read.csv(file = "D:/pddata.csv", head = TRUE)
# deleted columns of patients applied with placebo, which are recorded as NA
pkdata <- pkdata0[,-6]
pkdata <- pkdata[,-12]
pkdata <- pkdata[,-14]
pkdata <- pkdata[,-21]
pkdata <- pkdata[,-25]
pkdata <- pkdata[,-27]
pddata <- pddata0[,-6]
pddata <- pddata[,-12]
pddata <- pddata[,-14]
pddata <- pddata[,-21]
pddata <- pddata[,-25]
pddata <- pddata[,-27]

pkdata1 <- pkdata[,-1]
pddata1 <- pddata[,-1]

t <- pkdata[,1]
te <- pddata[,1]
k <- ncol(pkdata1)
# there are 6 main outputs: CFIT: fitted concentration: EFIT,:fitted effect (concentration of ANC); PKPARA(pk parameters):
```
```

```
k, and ka; PDPARA( pd parameters),:gamma, EC50; Cav, average concentration,; Eav, effect(or concentration of ANC)under Cav
# outputs for individuals are recorded by columns, statistics analysis can be applied to investigate some characteristics of sample or population
CFIT <- matrix(0,ncol = k, nrow = 601)
EFIT <- CFIT
PKPARA2 <- matrix(0,ncol = k,nrow = 4)
PKPARA <- matrix(0,ncol = k,nrow = 6)
PDPARA <- matrix(0,ncol = k,nrow = 4)
CAV <- matrix(0,ncol = k)
EAV <- CAV
# ====================================
# =part 2 pkpd parameter estimation=
# ====================================
# =pk=
# concentration - time curves approximately share the same trends under the same dosage, that is, for instance, when dosage is 30μg, all of the curves reach maximum value in 6th lag and begin to eliminate after 9th lag
# here, the shape of 4 dosages are restrained by given tmax and teli, which makes statistics for sample or population, such as mean and median, make sense. tmax is the time point that the concentration of drug reaching the maximum value, while, teli is the time point that drug beginning to eliminate.
for (i in 1:k) {
  if (i <= 5){
    teli <- 9
    tmax <- 6
  }else if (i <= 15 & i >= 6){
    teli <- 9
    tmax <- 6
  }else if (i <= 25 & i >= 16){
    teli <- 10
    tmax <- 8
  }else {
    teli <- 11
    tmax <- 8
  }
  # residual method is applied here, two - compartment model is chosen to fit the concentration data for each individual, output will be stored in matrix CFIT, parameters will be stored in matrix PKPARA
  # concentration data form 0 to maximum concentration and from maximum concentration to concentration beginning to eliminate are used to apply log-linear regression to estimate the pk parameters
  x <- pkdata1[,i]
  EFF <- pddata1[,i]
  # pk
  xtd1 <- x[teli:k]
```

```
76  td1 <- t[teli:k]
77  int1 <- lm(log(xtd1) ~ td1)$coefficients[1]
78  sl1 <- lm(log(xtd1) ~ td1)$coefficients[2]
79  res1 <- exp(int1 + sl1 * t)
80  xtd2 <- x[tmax:(teli-1)] - res1[tmax:(teli-1)]
81  td2 <- t[tmax:(teli-1)]
82  int2 <- lm(log(abs(xtd2)) ~ td2)$coefficients[1]
83  sl2 <- lm(log(abs(xtd2)) ~ td2)$coefficients[2]
84  res2 <- exp(int2 + sl2 * t)
85  xtd3 <- x[1:(tmax-1)] - res2[1:(tmax-1)] - res1[1:(tmax-1)]
86  td3 <- t[1:(tmax-1)]
87  int3 <- lm(log(abs(xtd3)) ~ td3)$coefficients[1]
88  sl3 <- lm(log(abs(xtd3)) ~ td3)$coefficients[2]
89  a <- exp(int2)
90  b <- -sl2
91  c <- exp(int1)
92  d <- -sl1
93  e <- -exp(int3)
94  f <- -sl3
95  k21 <- (a*d*(f-b)+c*b*(f-d)) / (a*(f-b)+c*(f-d))
96  k01 <- f/2
97  k10 <- b*d/k21
98  k12 <- b + d - k21 - k10
99  coeff <- c(a,b,c,d,e,f)
100 names(coeff) <- c("a","b","c","d","e","f")
101 para <- c(k01,k10,k12,k21)
102 names(para) <- c("k01","k10","k12","k21")
103 out <- list(coeff,para)
104 names(out) <- c("coeff","para")
105 tfit <- seq(0:600) - 1
106 cfit <- a * exp(-b * tfit) + c *exp(-d *tfit) + e *exp(-f *tfit)
107 cfit[1] <- 0
108 # ==================
109 # =pd=
    # biophrase is introduced here in order to fit the lag between concentration and effect, or concentration of ANC, ke0 describes
110 the transfer velocity, which is very close to ka, which can be computed by trails, in R, function Optim() is good for users to
    find ke0, in convenience, ke0 is set by 0.02, that is, the mean of ka in this study
111 # when ke0 is set, gamma and EC50 can be estimated by log-linear regression as well
112 ke0 <- 0.015
113 Emax <- max(EFF) * 1.05
114 E0 <- min(EFF) * 0.95
115 Em <- Emax - E0
```

```
    # log - linear regression
    NE <- (Em - (EFF - E0)) / (EFF - E0)
    Ce <- ke0*exp(-ke0*te)*(a/(ke0-b)*(exp((ke0-b)*te)-1) + c/(ke0-d)*(exp((ke0-d)*te)-1) + e/(ke0-f)*(exp((ke0-f)*te)-1))
    Ce[1] <- 0.05
    vec <- c(8:13)
    int <- lm(log(NE)[vec] ~ log(Ce)[vec])$coefficients[1]
    sl <- lm(log(NE)[vec] ~ log(Ce)[vec])$coefficients[2]
    gamma <- -sl
    EC50 <- exp(int/gamma)
    pdpara <- c(EC50,gamma,E0,Emax)
    cefit <- ke0*exp(-ke0*tfit)*(a/(ke0-b)*(exp((ke0-b)*tfit)-1) + c/(ke0-d)*(exp((ke0-d)*tfit)-1) +
e/(ke0-f)*(exp((ke0-f)*tfit)-1))
    cefit[1] <- 0.05
    efit <- E0 + (Em * cefit^gamma) / (EC50^gamma + cefit^gamma)
    # ========================


    # =related statistics, Cav and Eav=


    # AUC, according to the definition, AUC can be given by sum of fitted value of concentration
    AUC <- sum(cfit)
    # Cav, AUC / time, here time is set by 120, according to the design of experiment
    Cav <- AUC / 120
    # Eav is the effect( or concentration of ANC), related to Cav, in order to find Eav, 3 steps are needed, find time tav, find
concentration of biophrase ceav, find effect of average concentration Eav
    cdiff <- cfit - Cav
    targ <- abs(cdiff)
    tav <- match(min(targ[20:100]),targ)
    ceav <- ke0*exp(-ke0*tav)*(a/(ke0-b)*(exp((ke0-b)*tav)-1) + c/(ke0-d)*(exp((ke0-d)*tav)-1) +
e/(ke0-f)*(exp((ke0-f)*tav)-1))
    Eav <- E0 + (Em * ceav^gamma) / (EC50^gamma + ceav^gamma)
    # ========

    # =output=
    # ========
    CFIT[,i] <- t(cfit)
    EFIT[,i] <- t(efit)
    PKPARA[,i] <- t(coeff)
    PKPARA2[,i] <- t(para)
    PDPARA[,i] <- t(pdpara)
    CAV[i] <- Cav
    EAV[i] <- Eav
}
```

CFIT.REAL <- CFIT

```

# pk

```{r, message=FALSE,results="asis",echo=FALSE}
#==============
# =part 3 simulation=
# =============
# 4 parts are included:
# 1. fitted concentration - time curve of 4 dose groups , with comparison to original data;
# 2. fitted effect - time curve of 4 dose groups , with comparison to original data;
# 3. linear relationship between doses and related individual average concentration
# 4. relationship between average effect and $AUC_{0\sim120}$ of 4 dosage groups based on sigma Emax model
# doses for patients are known, then patients can be grouped by patient ID's
dose30 <- c(1:5)
dose60 <- c(6:15)
dose100 <- c(16:25)
dose200 <- c(26:30)
DOSE <- c(rep(30,5),rep(60,10),rep(100,10),rep(200,5))
d.d <- c(30,60,100,200)
# ========================
# concentration plot, individual fitted curves, average fitted curve and original data included, the average fitted curve is given by equation with mean value of parameters, in this way, the deviance of patients can be measured, furthermore, original data, fitted data and error are shown
```

dose30

dose30 is shown below as a example.

Change line 182 "dose<-dose30" and repeat line 182-215 if users want to compute parameters with other doses.

```{r, message=FALSE,results="asis",echo=FALSE}
dose <- dose30
pkp <- PKPARA[,dose]
pkp2 <- PKPARA2[,dose]
coeffp <- apply(pkp,1,mean)
coeffp2 <- apply(pkp2,1,mean)
stdp <- apply(pkp2,1,sd)
a <- coeffp[1]
b <- coeffp[2]
c <- coeffp[3]
d <- coeffp[4]
```
```

```
e <- coeffp[5]
f <- coeffp[6]
pkpfit <- a * exp(-b * tfit) + c *exp(-d *tfit) + e *exp(-f *tfit)
matplot(tfit,CFIT.REAL[,dose],type = "l", col = "black",xlim = c(0,120),ylim = c(0,80), ylab = "Concentration(ng/ml)", xlab = "time(h)")
lines(tfit,pkpfit,lwd = 2)
par(new = TRUE)
matplot(t,pkdata[,dose+1],pch = 20, col = "black",xlim = c(0,120),ylim = c(0,80),ylab = "Concentration(ng/ml)", xlab = "time(h)")

AUC <- sum(pkpfit[0:120])
mean.k <- coeffp2
names(mean.k) <- c("k01","k10","k12","k21")
sd.k <- stdp
names(sd.k) <- c("k01","k10","k12","k21")
data30p1 <- cbind(apply(pkdata[,dose+1],1,mean), pkpfit[t+1])
error <- (data30p1[,2] - data30p1[,1]) / data30p1[,1]
error[1] <- 0
data30p <- cbind(data30p1,error)
```

```{r}
AUC
mean.k
sd.k
data30p
```

```
# pd

## dose30 is shown below as an example.
Change line 226 "dose<-dose30" and repeat line 226-244 if users want to compute parameters with other doses.
```

```{r, message=FALSE,results="asis",echo=FALSE}
# ======
# effect plot, individual fitted curves and original data included, original data, fitted data and error are shown
dose <- dose30
matplot(tfit,EFIT[,dose],xlim = c(0,500), type = "l", ylim = c(0,40), ylab = "effect(10^9/L)", xlab = "time(h)", col = "black")
par(new = TRUE)
matplot(te, pddata1[,dose],xlim = c(0,500), ylim = c(0,40), pch = 20, ylab = "effect(10^9/L)", xlab = "time(h)", col = "black")

mean.gam <- apply(PDPARA[,dose],1,mean)
```

```
names(mean.gam) <- c("EC50","gamma","E0","Emax")
sd.gam <- apply(PDPARA[,dose],1,sd)
names(sd.gam) <- c("EC50","gamma","E0","Emax")

data30d1 <- cbind(apply(pddata1[,dose],1,mean),apply(EFIT[te+1,dose],1,median))
error <- (data30d1[,2] - data30d1[,1]) / data30d1[,1]
data30d <- cbind(data30d1,error)
```

```{r}
mean.gam
sd.gam
data30d
```

```{r, message=FALSE,results="asis",echo=FALSE}
# ======
# linear relationship between AUC0–120 and dose, weighted LSE are computed here
Q <- function(par,x,y) {
    x1 <- par[1] + par[2]*x
    x2 <- c(rep(x1[1],5),rep(x1[2],10),rep(x1[3],10),rep(x1[4],5))
    s <- sum(abs(x2 - y)/x2)
    return(s)
}
par0 <- optim(c(-1,1),Q,x = d.d, y = 120*CAV)
par <- par0$par
xa <- c(0:200)
ya <- par[1] + par[2]*xa
plot(DOSE,120*CAV,pch = 20,xlab = "dose(μg/kg)",ylab = "AUC(h*10^9/L)", xlim = c(30,200))
lines(xa[30:200],ya[30:200])

# ==========================
# sigmoid Emax model on average effect and dose, sigma E-max model and log-linear regression are used
d.e <- c(mean(EAV[dose30]), mean(EAV[dose60]), mean(EAV[dose100]), mean(EAV[dose200]))

cav30m <- mean(CAV[dose30])
cav30sd <- sd(CAV[dose30])
cav60m <- mean(CAV[dose60])
cav60sd <- sd(CAV[dose60])
cav100m <- mean(CAV[dose100])
```

```
cav100sd <- sd(CAV[dose100])
cav200m <- mean(CAV[dose200])
cav200sd <- sd(CAV[dose200])

eav30m <- mean(EAV[dose30])
eav30sd <- sd(EAV[dose30])
eav60m <- mean(EAV[dose60])
eav60sd <- sd(EAV[dose60])
eav100m <- mean(EAV[dose100])
eav100sd <- sd(EAV[dose100])
eav200m <- mean(EAV[dose200])
eav200sd <- sd(EAV[dose200])
m1 <- rbind(cav30m,cav60m,cav100m,cav200m)
sd1 <- rbind(cav30sd,cav60sd,cav100sd,cav200sd)
cav <- cbind(m1,sd1)
m2 <- rbind(eav30m,eav60m,eav100m,eav200m)
sd2 <- rbind(eav30sd,eav60sd,eav100sd,eav200sd)
eav <- cbind(m2,sd2)

emax <- max(d.e) * 1.05
e0 <- 0
em <- emax - e0
Y <- (em - (d.e - e0)) / (d.e - e0)
X <- m1
int <- lm(log(Y) ~ log(X))$coefficients[1]
sl <- lm(log(Y) ~ log(X))$coefficients[2]
gamma1 <- -sl
EC501 <- exp(int/gamma1)
cefit1 <- c(0:500)
efit1 <- e0 + (em * cefit1^gamma1) / (EC501^gamma1 + cefit1^gamma1)
plot(X,d.e,pch = 22, xlab = "concentration(ng/ml)", ylab = "effect(10^9/L)", xlim = c(0,250), ylim    = c(20,30))
lines(cefit1,efit1)
EMAX <- efit1[500]
EC90 <- exp((1/9)^(-gamma1))
# ======================
# =simulation results=
# ======================
#d.e
#X
```

```
#CFIT
#CFIT.REAL
#EFIT
#PKPARA
#PKPARA2
#PDPARA
```

```

## Average concentration and average effect

```{r}
cav
eav
EMAX
EC90
```

```

# Goodness of Fit

```{r, message=FALSE,results="asis",echo=FALSE}
# ====================
# =part 4 model diagnostics=
# ====================
dose <- dose30 #
CFIT1 <- CFIT[t+1,dose]
pk1 <- pkdata1[,dose]
mpk <- apply(pk1,2,mean)
dm.c <- (CFIT1 - mpk)^2
do.c <- (pk1 - CFIT1)^2
dif <- pk1 - CFIT1
SE.c <- apply(dif,2,sd)
SST.c <- apply(dm.c,2,sum)
SSE.c <- apply(do.c,2,sum)
SSR.c <- SST.c - SSE.c
R2.c <- SSR.c / SST.c
R2.adj <- 1 - (1 - R2.c) * 16 / 10
AIC.c <- 12 + 17 * log(SSE.c)
BIC.c <- 17 * log(SSE.c) + 6 * log(17)
#====================================
EFIT1 <- EFIT[te+1,dose]
pd1 <- pddata1[,dose]
mpd <- apply(pd1,2,mean)
dm.e <- (EFIT1 - mpd)^2
do.e <- (pd1 - EFIT1)^2
dif <- pd1 - EFIT1
SE.e <- apply(dif,2,sd)
SST.e <- apply(dm.e,2,sum)
SSE.e <- apply(do.e,2,sum)
SSR.e <- SST.e - SSE.e
R2.e <- SSR.e / SST.e
R2.adj <- 1 - (1 - R2.e) * 17 / 14
AIC.e <- 6 + 18 * log(SSE.e)
BIC.e <- 18 * log(SSE.e) + 3 * log(18)
```

```

## PK

```{r}
R2.c
AIC.c
BIC.c
```

```

## PD

```{r}
R2.e
AIC.e
BIC.e
```

```

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