Pharmacodynamic Modeling of Prednisolone Effects on Natural Killer Cell Trafficking

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INTRODUCTION

Corticosteroids, such as prednisone, have been widely used to treat a variety of conditions requiring their hormonal, anti-inflammatory and immunosuppressive effects. It is well documented that corticosteroids alter the circulating population of leukocytes such as basophils (1) and T-helper cells (2). These pharmacodynamic effects have been characterized as corticosteroid inhibition of these cells entering blood from extravascular sites (2,3). Similar approaches have been also applied to describe the pharmacodynamic effects of corticosteroids on cortisol suppression (4) and osteocalcin suppression (5).

Natural killer (NK) cells are defined functionally by their ability to lyse target cells without deliberate prior sensitization and without restriction by major histocompatibility antigens (6). Such activity was first described in the 1970's when it was observed that lymphocytes freshly isolated from normal nonimmunized hosts could lyse allogenic tumor cell lines (7,8). NK cells comprise 10-15% of human peripheral blood lymphocytes and are large granular lymphocytes (9). They have been implicated in several activities in vivo, including destruction of tumor cells, resistance to viral infections, and regulation of hematopoiesis (10,11).

NK cells contain glucocorticoid receptors which bind dexamethasone (K_D = 6.3 nM) similar to monocytes and neutrophils (12). Kats et al. (13) studied the effects of hydrocortisone on circulatory kinetics of NK cells in humans as well as the lymphocyte-mediated cytotoxicity. They observed that the blood population of NK cells was elevated after hydrocortisone administration; however, this effect was maintained only temporarily with a return to pretreatment levels by 24 h after dosing, and NK cytotoxicity also followed the similar pattern. Field et al (14) also observed a similar trafficking pattern of NK cells during an exercise and recovery phase. One possible explanation of this exercise-related NK cell trafficking was that exercise can increase plasma cortisol level which would result in the net movement of NK cells into the blood pool. During the recovery phase, both plasma cortisol and NK cells eventually returned to baseline (15).

So far, pharmacodynamic models have not yet been utilized to describe this corticosteroid action. Previously we proposed four basic models of indirect pharmacodynamic responses (16). We also showed the applicability of the models to several actual pharmacodynamic responses (17). In this report, we first quantitate the effects of prednisolone on the trafficking of NK cells using two possible indirect pharmacodynamic models.

METHODS

Twelve normal volunteers were given 10 mg/day of either prednisone or placebo tablets for seven days with a four week washout period between treatments. Serial blood samples for determination of the prednisolone concentrations and the blood population of NK cells were collected just prior to and at 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 h after the first and last (day 7) dose of either prednisone or placebo tablets. Prednisone is a widely used prodrug of its active metabolite, prednisolone; these compounds show reversible metabolism and the plasma concentrations of prednisone and prednisolone are similar regardless of which one was administered (18). The pharmacokinetics of prednisolone was used in tandem with the pharmacodynamics of NK cell trafficking after prednisone was given.

Prednisolone concentrations were measured in plasma by microbore HPLC (19) with a limit of quantitation of 5 ng/ml. Absorption/conversion and disposition according to a one-compartment model was described by:

$$C_p = \frac{k_a F D}{V (k_a - k_e)} (e^{-k_e t} - e^{-k_a t}) \quad (1)$$

where D is dose, k_a is the absorption rate constant, k_e is the elimination rate constant, and V/F is the volume of distribution/availability of the drug. First-order oral absorption without a lag-time was assumed. Least-squares regression was performed using the PCNONLIN program (SCI Software Inc., Lexington, KY) with $1/C_p$ weighting. The pharmacokinetic parameters for each person were used to produce the input of plasma concentrations of prednisolone for pharmacodynamic responses at corresponding time points.

Total leucocyte counts were performed using an automated hemocytometer (Coulter Counter S. Plus IV; Coulter Electronics Inc., Hialeah, FL) on whole blood samples. Lymphocyte and monocyte ratios were determined microscopically, and the total number of circulating lymphocytes per cubic millimeter was determined. The whole blood samples are then lysed, reacted with mononuclear antibody (Coulter Cytostat anti-CD3, CD4, and CD8, FITC), and analyzed on an automated flow cytometer (FACS 440; Becton Dickinson Co). Multiplication of the proportion of fluorescent cells by the number of circulating lymphocytes resulted in the total number of circulating NK cells (CD3−, CD4−, CD8+). Blood profiles of NK cells in the placebo group (day 1 and day 7) were characterized as the baselines.

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The pharmacodynamic models are structured with blood and extravascular compartments accounting for cell trafficking (Fig. 1).

The baseline data are thus described by:

$$\frac{dNK_B}{dt} = k_{in}^o - k_{out}\, NK_B \quad (2)$$

where NK_B represents the blood population of NK cells during placebo treatment, k_{in}^0 is the zero-order input rate constant to describe NK cells migrating into blood from extravascular sites, and k_{out} is the first-order rate constant for NK cells egress from blood.

Two related approaches among four basic indirect response models (16,17), were examined for changes in NK cells during prednisolone treatment (NK_p), namely stimulation of NK cell entry into blood (Fig. 1 Model S) and inhibition of cell efflux from blood (Fig. 1 Model I).

Stimulation Model

The stimulation (S) model can be described as follows:

$$S(t) = 1 + \frac{E_{max}\, C_p}{SC_{50} + C_p} \quad (3)$$

$$\frac{dNK_p}{dt} = k_{in}^0\, S(t) - k_{out}\, NK_p \quad (4)$$

S(t) is the function describing the stimulation effect of prednisolone on the trafficking of NK cells where E_{max} and SC_{50} represent the capacity and affinity constants. The prednisolone concentrations (C_p) at corresponding times were calculated by applying pharmacokinetic parameters from each subject (k_a, k_e, V/F) to equation 1. The value of k_{out} can be calculated as:

$$k_{out} = \frac{k_{in}^0}{NK_{ss}} \quad (5)$$

where NK_{ss} is the value of NK cells at steady-state during the corresponding baseline phase. Initial estimations of E_{max}, SC_{50}, and k_{in}^0 for model S were calculated. As presented in equation 3, S(t) is bound between 1 (when $C_p \rightarrow 0$) and $1 + E_{max}$ (when $C_p \gg SC_{50}$ at early times). At $T_{max}(S)$, the prednisolone concentration is still much higher than the value of SC_{50}; therefore, equation 4 can be written as:

$$\frac{dNK_p}{dt} = k_{in}^o\,(1 + E_{max}) - k_{out}\, NK_p^{max} = 0 \quad (6)$$

By rearranging equation 6, we obtain

$$k_{in}^o\,(1 + E_{max}) = k_{out}\, NK_p^{max} \quad (7)$$

and, upon substitution of equation 7 for k_{out}, yields:

$$E_{max} = \frac{NK_p^{max}}{NK_{ss}} - 1 \quad (8)$$

The value of SC_{50} was initially estimated by graphically determining the time when 50% of the maximum NK cells (NK_p^{max}) occurred in the rising phase during the trafficking process; and then calculating the prednisolone concentration at the corresponding time by applying equation 1. The k_{in}^0 for model S was estimated by the equation:

$$k_{in}^o\,(S) = \frac{(NK_p^{max} - NK_{ss})}{T_{max}(S)\,(1 + E_{max})} \quad (9)$$

Inhibition Model

The equations for the inhibition (I) model are:

$$I(t) = 1 - \frac{C_p}{(C_p + IC_{50})} \quad (10)$$

$$\frac{dNK_p}{dt} = k_{in}^o - k_{out}\, I(t)\, NK_p \quad (11)$$

I(t) is the inhibition function describing the effect of prednisolone on the trafficking of NK cells where IC_{50} is the steroid concentration at which k_{out} is diminished by 50%. Prednisolone concentrations were calculated in the same way as used for model S. The value of IC_{50} for model I was initially estimated by graphically determining the time when 50% of the maximum NK cells (NK_p^{max}) occurred in the decline phase during the trafficking process; and then, calculating the prednisolone concentration at the corresponding time by applying equation 1. The k_{in}^0 for model I was calculated as

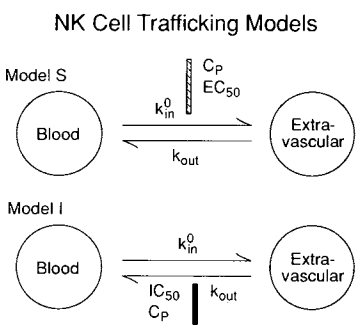

Fig. 1. Diagrammatic representative of the NK cell trafficking models in which k^0_{in} is the zero-order constant describing the rate of NK cells entering the blood compartment, k_{out} is the first-order rate constant for the movement of NK cells from blood to extravascular sites, C_p is the plasma prednisolone concentration, EC_{50} is the prednisolone concentration producing a 50% increase in k_{in}^0 (model S), IC_{50} is the prednisolone concentration producing a 50% decrease in k_{out} (model I).

Table I. Pharmacokinetic Parameters of Prednisolone in 12 Volunteers (mean ± SD)

Parameters	Day 1	Day 7
C_{max}, ng/ml	138.7 ± 32.3	127.0 ± 36.7
t_{max}, h	1.02 ± 0.66	1.38 ± 1.00
V/F, L	53.7 ± 12.7	55.5 ± 12.9
k_a, h^{-1}	4.45 ± 3.67	4.12 ± 3.94
k_{el}, h^{-1}	0.310 ± 0.084	0.276 ± 0.078
$t_{1/2}$, h	2.37 ± 0.57	2.68 ± 0.7
CL/F, L/h	16.0 ± 3.39	14.7 ± 2.9
AUC, ng · h/ml	647 ± 111	703 ± 133

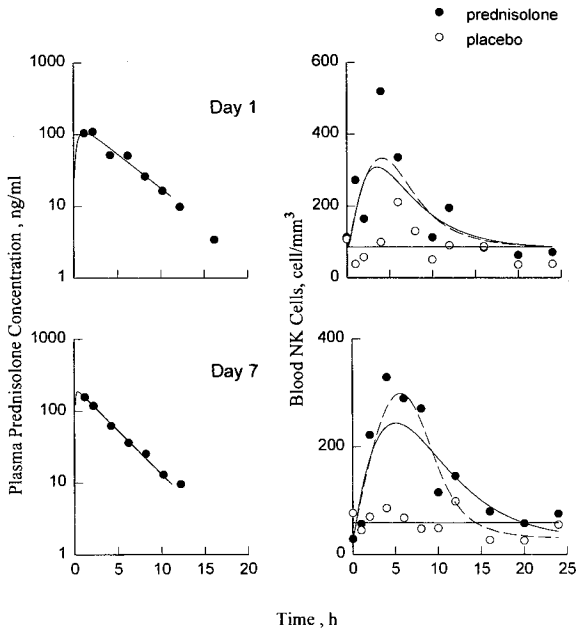

Fig. 2. Representative graph describing plasma prednisolone concentrations and blood NK cell numbers as a function of time following prednisone administration and placebo treatment for a single dose (day 1) and multiple doses (day 7) at a dose of 10 mg/day for subject 7. The lines were generated by nonlinear least-squares regression to fit the model to corresponding data. Solid line: model S; Dashed line: model I.

$$k_{in}^{o}(I) = \frac{(NK_p^{max} - NK_{ss})}{T_{max}(I)} \quad (12)$$

where $T_{max}(I)$ is the time when the peak level of NK cells has been measured experimentally. k_{out} was calculated by equation 5.

The appropriate sets of equations (1, 3, 4 for model S, 1, 10, 11 for model I) were fitted simultaneously to the NK cell numbers obtained from prednisolone treatments using the PCNONLIN program with no weighting of data. The NK cell data of placebo treatment group were supposed to be identical with the NK_{ss} of the prednisolone treatment group, but some intrasubject variation occurred. In order to get the best fitted curves, arithmetic means of NK cell data were used as a NK_{ss} for the placebo treatment group. Even though the data from day 1 and day 7 came from the same individual, the models were fitted to them separately because the NK_{ss} values were not close enough to get the best simultaneous fitting.

RESULTS AND DISCUSSION

Prednisone was rapidly absorbed and metabolized to its active metabolite, prednisolone, which was indicated by the rapid initial rise in prednisolone plasma concentrations. The elimination was generally monoexponential. Some curves showed late phases which were suggestive of biexponential disposition, but it was difficult to consistently fit these terminal phases. The pharmacokinetic parameters obtained using the one-compartment model are shown in Table I. There was no significant difference between mean AUC values from day 1 and day 7. Thus, in case of multiple-doses of prednisone, previous exposure does not appear to affect the pharmacokinetics of the last dose. The fitted k_a, k_e, V/F values were used to produce the corresponding input of plasma drug concentrations for the pharmacodynamic responses.

The baseline concentration of NK cells was maintained constant following placebo treatment. With oral prednisone, the NK cells in blood gradually increased and reached its peak value at approximately 4 h, then cell numbers slowly returned to baseline level at about 16 h post-dosing (Fig. 2). Both of the models were able to characterize the general pattern of NK cell trafficking after single (day 1) and multiple doses (day 7). However, a better fitting for the maximum was obtained when the model I was applied, especially for the subject most sensitive to the drug (Fig. 2).

The mean ratio of control NK cell numbers among 12 subjects (day 1/day 7) was 1.235 ± 0.447, indicating no influence of multiple dosing of prednisolone on NK cell trafficking.

The mean pharmacodynamic parameters of the two models are presented in Table II. The SC_{50} and IC_{50} values for each subject are shown in Fig. 3 as representative pharmacodynamic parameters for each model. These parameters varied depending on individuals and days. However, both IC_{50} and SC_{50} values exhibited similar variability among subjects.

Prednisolone shows nonlinear protein binding from 95% at lower concentrations (<100 ng/ml) to 60% at higher concentrations (>800 ng/ml). However, total and free prednisolone concentrations provided equivalent characterization of cortisol, basophil, and T-helper cell profiles when supplied to pharmacodynamic models (20). Thus, only total drug concentrations were used for these pharmacodynamic models.

In order to further examine the characteristics of these two models, simulations of the effects of increasing steroid doses were conducted. All parameters (both pharmacokinetic and pharmacodynamic) were maintained constant, except

Table II. Pharmacodynamic Parameters for Prednisolone Effects on NK Cell Trafficking (mean ± SD)

Parameters	Stimulation model		Inhibition model	
	Single dose	Multiple dose	Single dose	Multiple dose
k_{out}, h^{-1}	0.255 ± 0.182	0.205 ± 0.111	0.623 ± 0.619	0.632 ± 0.778
k^0_{in}, cell/(mm^3 · h)	12.6 ± 11.8	8.6 ± 4.9	34.4 ± 46.0	23.0 ± 22.5
IC_{50}, ng/ml	NA	NA	27.6 ± 26.3	50.3 ± 61.5
E_{max}	15.4 ± 18.5	17.22 ± 20.81	NA	NA
SC_{50}, ng/ml	518 ± 645	503 ± 587	NA	NA

NA: not applicable.

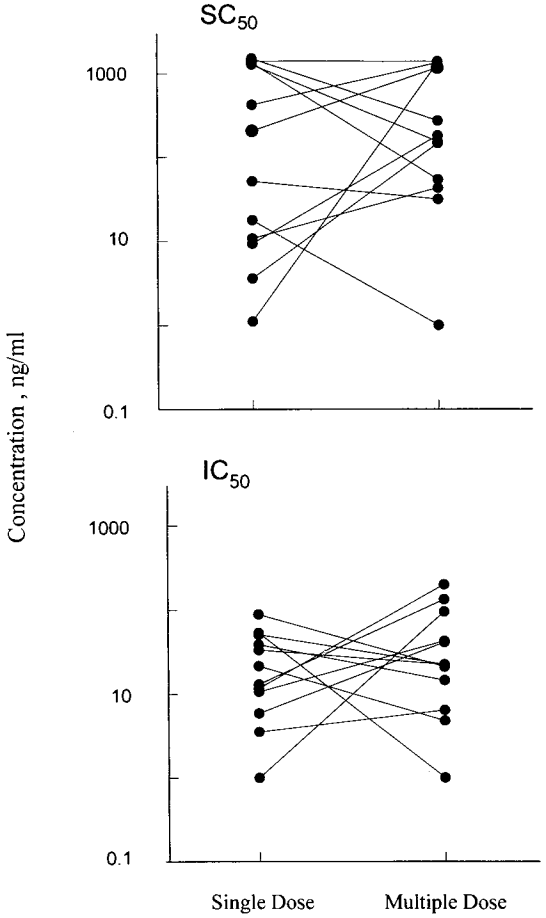

Fig. 3. SC_{50} and IC_{50} values of prednisolone for each subject obtained from the model S and model I for single dose (day 1) and multiple doses (day 7) at a dose of 10 mg/day.

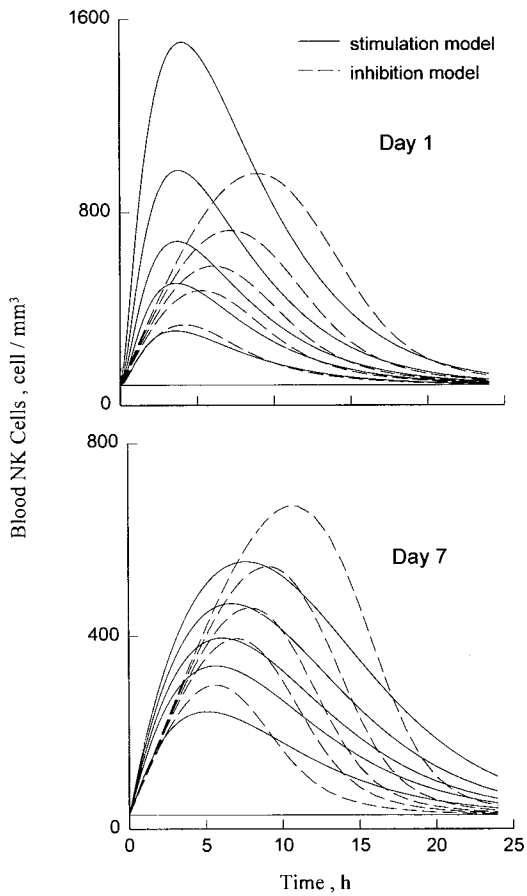

Fig. 4. Representative simulation graph of the population of blood NK cells versus time following prednisolone at doses of 10, 20, 30, 50, and 100 mg/day for subject 7.

for the dose (10, 20, 30, 50, and 100 mg/day). Parameters for subject 7, day 1 were selected as representative. The results of the simulation are presented in Fig. 4. For both models, increases in dose produce higher areas under the effect curves (AUEC). There is a comparable linear relationship between the dose and AUEC for both models. The respective slopes, intercepts, and correlation coefficients are 151.7, 2021, 0.98 ($p < 0.05$) for model S and 112.8, 2438, 0.94 ($p < 0.05$) for model I.

The rate of onset of changes in NK cell number attains a maximum value as it is bound to k_{in}^0 for model I, and $k_{in}^0 \cdot (1 + E_{max})$ for model S. The times of maximum response (T_{max}) of the model S and I were shifted to the right with increasing doses, although the shift is not graphically noticeable in case of the model S (day 1). In the evaluation of the effect of 0 to 100 mg doses of methylprednisolone on the increase of segmented granulocytes in blood, the data of Derendorf et al. (21), though not very time intensive, suggest that larger doses cause a shift of the T_{max} to later times as expected from both models S and I.

At the present time, we are not able to determine which model is more suitable on the basis of curve fitting as there are no data available to examine the pattern of NK cell trafficking at higher doses. However, model S has a disadvantage of requiring the generation of two dynamic parameters (E_{max} and SC_{50}) as opposed to the more parsimonious model I where only IC_{50} needs to be fitted for (in addition to the k_{in}^0 value which is needed for both models). The mean IC_{50} values for NK cell trafficking in model I (27.6 ng/ml for day 1, 50.3 ng/ml for day 7) are similar to the IC_{50} values for the other prednisolone effects (10.3 ng/ml for cortisol suppression, 48.8 ng/ml for basophil trafficking, 120 ng/ml for T-cell trafficking) (20). For other cell trafficking effects in the rat, it has been shown that the steroids inhibit efflux of lymphocytes from organs/tissues of spleen, lymph nodes and bone marrow (22). Thus the inhibitory mechanism and model I appear, at present, to be most relevant to the trafficking dynamics of NK cells.

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