Research Article

Assessment of the Impact of Dosing Time on the Pharmacokinetics/Pharmacodynamics of Prednisolone

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Abstract. Prednisolone is widely used for the treatment of inflammation and auto-immune diseases. It exhibits nonlinear pharmacokinetics (PK); and its induced systemic effects (pharmacodynamics (PD)) are commonly evaluated with two biomarkers, cortisol and blood lymphocytes in plasma. Circadian patterns are observed in both biomarkers. Furthermore, the disease itself may show a circadian pattern. For example, in rheumatoid arthritis patients, better therapeutic outcomes have been reported when prednisolone was administered in the very early morning. The aim of this study is to evaluate the impact of dosing time on the PK/PD of prednisolone with a simulation approach using an interactive algorithm. A series of simulations were performed with either intravenous or oral administration of prednisolone or prednisone. The results showed that the initial or maximum concentration and trough concentration of total prednisolone were lower when the drug was administered in the early morning around 6 AM. Oscillation patterns were observed in cumulative cortisol suppression (CCS) and alteration of total lymphocyte trafficking in blood. When the drug was given in the morning within the therapeutic dose range, or around 6 PM for a small dose amount (<1 mg), the minimum CCS and maximum effect on lymphocytes were observed. These results indicated that the PK/PD of prednisolone are time- and dose-dependent, and suggested that it is necessary to consider the application of chronotherapy to achieve better clinical outcomes with fewer side effects of prednisolone, and a PK/PD simulation approach could provide a valuable tool to evaluate and predict time-dependency in the system.

KEY WORDS: dosing time; pharmacokinetics/Pharmacodynamics; prednisolone; simulation.

INTRODUCTION

Time-dependency of biological and physiological processes, such as rhythms of blood pressure, enzyme expression, immune cell production, and endocrine functions, has been recognized in most mammalian systems. Based on this principle, chronotherapy was developed, and has been applied in several disease areas, including cancer, immune diseases, and cardiovascular diseases (1–4). Chronotherapy is implemented to achieve better clinical outcomes with fewer side effects by choosing an optimal dosing time. It is not surprising that drug pharmacokinetics (PK) and pharmacological effects are perhaps time-dependent of dosing.

Corticosteroids are widely used in the treatment of allergies, inflammation and autoimmune diseases, such as asthma, lupus, rheumatoid arthritis (RA), and Crohn's disease. Generally, corticosteroids are given in the morning due to the disease related process. For example, patients with RA experience more clinical symptoms during the early morning, which is believed to be associated with the peak level of interleukin 6 (IL-6) (5). However, cells and cytokine

levels in immune system show circadian variations, including T lymphocytes (6), and IL-6 (5,7). And corticosteroids could potentially alter the rhythmic variation in the immune system. Therefore, it is important to understand the impact of dosing time on the pharmacological effects of corticosteroids. When prednisolone was administered in the very early morning hours, better therapeutic outcomes have been observed in RA patients (8). When prednisolone was given afternoon at 4 PM, duration of the suppression of cortisol in plasma was shortened compared with 8 PM treatment (9). Recently, Glassmarmor and colleagues found that patients with multiple sclerosis preferred night-time treatment of methylprednisolone, and higher clinical recovery was observed for night-time treatment (2). In a simulation study, Krishnaswami *et al* found that dosing of inhaled steroids at around 3 to 4 AM gave the maximum 24-h cumulative cortisol suppression (CCS) in plasma, and minimum CCS occurred in the afternoon around 4 to 7 PM (10). These results indicated that clinical outcomes and side effects could be influenced by the time of administration of corticosteroids.

The exogenous corticosteroid, prednisolone, has been used systemically for several decades (11). Total prednisolone in plasma showed nonlinear PK in several species, including humans (12–17). Prednisolone, like other corticosteroids, mediates several important pharmacological effects, including immune-suppression, and cortisol suppression. So far, however, the time-dependency of the PK and pharmacodynamics

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Fig. 1. Pharmacokinetic/Pharmacodynamic (PK/PD) model of prednisone and prednisolone. One-way arrows with dash lines, the protein binding; one-way arrows with square dotted lines, PK/PD interactions; the big two-way arrow, the competitive protein binding between cortisol and prednisolone. The model structure and symbols are described in the text

(PD) has not been assessed to help improve the clinical use of prednisolone. Recently, we developed a PK/PD approach to predict total prednisolone concentrations in human plasma, and this approach adequately explained the nonlinear prednisolone PK by combining nonlinear protein binding of prednisolone and other factors, including reversible metabolism between prednisolone and prednisone, prednisolone-induced cortisol suppression, and cortisol circadian rhythm (18). In this report, we propose to use this PK/PD approach to investigate time-dependency of prednisolone PK, and extend this PK/PD approach to evaluate the impact of dosing time on PD. Two major biomarkers, cortisol concentration and lymphocyte trafficking in plasma, are monitored for the systemic activities of prednisolone. Cortisol suppression was commonly modeled with the cortisol circadian and a suppression effect on cortisol release by exogenous corticosteroids (19,20). Lymphocyte trafficking was modeled with inhibition effects by both endogenous cortisol and exogenous corticosteroids (21,22). An interactive algorithm based on our PK/PD approach and other literature information was developed (18,21). Several predictions were performed to assess the validity of this algorithm *via* comparing simulated results with literature reported data. In this algorithm, a series of simulations with various dosing time and dose amounts were conducted with either intravenous administration of prednisolone or oral administration of prednisone. The simulated results, including initial, maximum, and trough total prednisolone concentrations, CCS and altered lymphocyte trafficking, were evaluated for the time-dependency of the PK/PD of prednisolone.

METHODS

Pharmacokinetics/Pharmacodynamics of Prednisolone/Prednisone

Pharmacokinetics of Prednisolone/Prednisone (18)

A linear two-compartmental PK model, previously developed in this lab, was used to describe free prednisolone

and free prednisone concentration-time profiles. Briefly, free prednisolone and free prednisone are one-compartmental with a first-order elimination process from each compartment. A simple linear interconversion is assumed between free prednisolone and free prednisone (In Fig. 1, shadow area, and Eqs. 1–2).

$$V_{PN} \cdot \frac{dC_{free}^{PN}}{dt} = CL^{PNL \rightarrow PN} \cdot C_{free}^{PNL} - (CL^{PN} + CL^{PN \rightarrow PNL}) \cdot C_{free}^{PN} \quad (1)$$

$$V_{PNL} \cdot \frac{dC_{free}^{PNL}}{dt} = CL^{PN \rightarrow PNL} \cdot C_{free}^{PN} - (CL^{PNL} + CL^{PNL \rightarrow PN}) \cdot C_{free}^{PNL} \quad (2)$$

Where C_{free}^{PN} and C_{free}^{PNL} are free concentrations of prednisone and prednisolone in each compartment; $CL^{PNL \rightarrow PN}$ and $CL^{PN \rightarrow PNL}$ are conversion clearances between free prednisolone and prednisone; CL^{PNL} and CL^{PN} are clearances of free prednisolone and prednisone; V_{PNL} and V_{PN} are volumes of distribution of free prednisolone and prednisone, respectively. When prednisolone or prednisone is given orally, their absorption processes are assumed to be first-order.

Total prednisolone concentrations, influenced by competitive protein binding from cortisol, are obtained based on a combination of Langmuir and linear type protein binding. Total prednisone concentrations are predicted with a linear type protein binding (Eqs. 3–4).

$$C_{total}^{PNL} = \frac{N_t \cdot P_t \cdot K_t^{PNL} \cdot C_{free}^{PNL}}{1 + K_t^{PNL} \cdot C_{free}^{PNL} + K_t^{COR} \cdot C_{free}^{COR}} + N_a \cdot P_a \cdot K_a^{PNL} \cdot C_{free}^{PNL} + C_{free}^{PNL} \quad (3)$$

$$C_{total}^{PN} = C_{free}^{PN} / f_{free}^{PN} \quad (4)$$

Where C_{total}^{PN} and C_{total}^{PNL} are total concentrations of prednisone and prednisolone in plasma; N_t , P_t , and K_t are total number of binding sites per transcortin, plasma transcortin concentration, and transcortin affinity constant; N_a , P_a , and K_a are total number of binding sites per albumin, plasma albumin concentration, and albumin affinity constant; f_{free}^{PN} is the free fraction of prednisone in plasma; C_{free}^{COR} is free cortisol concentration in plasma, which is related to C_{total}^{COR} as described in Eq. 5.

$$C_{total}^{COR} = \frac{N_t \cdot P_t \cdot K_t^{COR} \cdot C_{free}^{COR}}{1 + K_t^{COR} \cdot C_{free}^{COR} + K_t^{PNL} \cdot C_{free}^{PNL}} + N_a \cdot P_a \cdot K_a^{COR} \cdot C_{free}^{COR} + C_{free}^{COR} \quad (5)$$

Pharmacodynamics of Prednisolone/Prednisone

Cortisol Suppression by Prednisolone/Prednisone (18,20)

Cortisol circadian rhythm as well as cortisol suppression by prednisolone was described by a linear release model with an inhibition effect (In Fig. 1, right part, Eqs. 6–8).

$$\frac{dC_{total}^{COR}}{dt} = R^{COR} \cdot \left(1 - \frac{I_{max}^{PNL} \cdot C_{free}^{PNL}}{IC_{50}^{PNL} + C_{free}^{PNL}}\right) - k_e^{COR} \cdot C_{total}^{COR} \quad (6)$$

$$R^{COR} = \frac{R_{max}}{V_{COR} \cdot (t_{max} - t_{min} - 24)} \cdot (t - t_{min}) \quad (7)$$

$(t_{max} \leq t \leq t_{min})$

$$R^{COR} = \frac{R_{max}}{V_{COR} \cdot (t_{max} - t_{min})} \cdot (t - t_{min}) \quad (t_{min} \leq t \leq t_{max}) \quad (8)$$

Where R^{COR} , cortisol release rate, is described with two linear functions based on the time; and k_e^{COR} is the first-order elimination rate constant; I_{max}^{PNL} and IC_{50}^{PNL} are the maximum inhibition effect by prednisolone and free prednisolone concentration to produce 50% of maximum suppression; and R_{max} is the maximum cortisol release rate at time t_{max} .

Cumulative cortisol suppression (CCS) within 24 h is commonly used to assess prednisolone systemic activities. CCS is based on the difference between the area under cortisol baseline curve ($AUC_{baseline}^{COR}$) and the area under curve of plasma cortisol concentrations after prednisolone exposure ($AUC_{Treatment}^{COR}$) (Eq. 9). The area under curve is calculated using the trapezoidal rule.

$$CCS\% = \frac{AUC_{baseline}^{COR} - AUC_{treatment}^{COR}}{AUC_{baseline}^{COR}} \cdot 100\% \quad (9)$$

Inhibition of Total Lymphocyte Trafficking by Prednisolone/Prednisone (21,22)

The lymphocyte count was normalized as a percentage of the total lymphocyte count at 8:00 AM. Total lymphocyte

trafficking was described by a first-order elimination (k_{out}^{LYM}) process, and a zero-order production (k_{in}^{LYM}) with inhibition effects from both endogenous cortisol and exogenous prednisolone (In Fig. 1, right part, Eq. 10).

$$\frac{dN}{dt} = k_{in}^{LYM} \cdot \left(1 - \frac{I_{max,LYM}^{COR} \cdot (C_{free}^{COR} + IC_{50,LYM}^{COR}) / IC_{50,LYM}^{PNL} \cdot C_{free}^{PNL}}{IC_{50,LYM}^{COR} + (C_{free}^{COR} + IC_{50,LYM}^{COR}) / IC_{50,LYM}^{PNL} \cdot C_{free}^{PNL}}\right) - k_{out}^{LYM} \cdot N \quad (10)$$

Where N is the percentage of the count at 8:00 AM; $I_{max,LYM}^{COR}$ is the maximum inhibition effect by cortisol; $IC_{50,LYM}^{COR}$ is free cortisol concentration to produce 50% of maximum inhibition; $IC_{50,LYM}^{PNL}$ is free prednisolone concentration to produce 50% of maximum inhibition, which was calculated from $IC_{50,LYM}^{COR}$ and the receptor binding affinity of cortisol and prednisolone.

Similar to CCS, altered total lymphocyte trafficking within 24 h is evaluated by difference between the area under the effect curve (AUEC) without prednisolone ($AUEC_{baseline}^{LYM}$) and AUEC after prednisolone exposure ($AUEC_{treatment}^{LYM}$) (Eq. 11). The area under curve is calculated using the trapezoidal rule.

$$AUEC\% = \frac{AUEC_{baseline}^{LYM} - AUEC_{treatment}^{LYM}}{AUEC_{baseline}^{LYM}} \cdot 100\% \quad (11)$$

Design of an Interactive Algorithm

Using the PK/PD models given above, an interactive algorithm was developed in Microsoft Excel®. This algorithm was based on a deterministic approach without incorporating any variance component. All PK/PD parameters in this algorithm were obtained from literature (18,21). Cortisol circadian rhythm and its suppression were solved in a similar manner as described by Krishnaswami *et al* (10). All the other ordinary differential equations (ODEs), including the PK of free prednisolone and free prednisone, and total lymphocyte trafficking, were solved by the classical 4th order Runge-Kutta method. Two interactive Excel spreadsheets were created for oral administration and intravenous administration, and may be accessed at (<http://www.cop.ufl.edu/safezone/pat/pc/Derendorf/TheAAPSJ-D-08-00016-2008/SUPPLEMENTS/2-Oral-Admin.xls>), and (<http://www.cop.ufl.edu/safezone/pat/pc/Derendorf/TheAAPSJ-D-08-00016-2008/SUPPLEMENTS/1-Intravenous-Admin.xls>) respectively. The spreadsheets are able to display PK and PD profiles in a real-time manner, and predict CCS% and AUEC% based on the input parameters: name of drug (prednisone or prednisolone), dose amount (mg), time of administration (24 h clock scale), dosing regimen (single or multiple), and dose interval (Tau, for multiple doses). The interface of the spreadsheets is shown in Fig. 2.

Fig. 2. Excel® interface for prediction of pharmacokinetics/pharmacodynamics of prednisolone/prednisone

Predictability of the Algorithm

The predictability of this algorithm was illustrated by comparing the simulated results to the literature data visually. PK predictability of this algorithm was presented in our recently published paper (18). In terms of PD predictability, cortisol baseline and lymphocyte trafficking baseline were simulated and plotted against data digitally extracted from several clinical studies (21–27). If absolute counts of total lymphocytes are reported, data were converted to the percentage of the count at 8:00 AM. Cortisol suppression and inhibition of lymphocyte trafficking after prednisone or prednisolone exposure were simulated. Simulated cortisol and lymphocyte profiles were plotted with the extracted data from literature (27–31). Predicted CCS% was also compared with CCS% calculated from literature (14,25,26,32). The choice of dose amounts and routes in simulation was dictated by clinical studies in literature.

Simulations in the Algorithm

Several simulations were performed to assess the impact of dosing time on the PK/PD of prednisolone in this algorithm. Scripts were created in Visual Basic Application (VBA®) to facilitate simulations.

Intravenous Administration of Prednisolone

Prednisolone is commonly administrated intravenously at 2 to 100 mg dose range. First simulation was conducted within this range at a 2 mg step increase. Both endogenous cortisol and exogenous prednisolone inhibit lymphocyte trafficking; in addition, exogenous prednisolone suppresses endogenous cortisol production; thus, a second simulation scenario within a dose range of 0.01 to 1 mg was performed to understand the net steroid effect on lymphocyte trafficking. Time of administration was chosen within a 24 h range at a 2 h interval. During simulations, initial concentrations of total prednisolone ($C_{(0)}$), CCS% and AUEC% were recorded.

Contour plots were created with dose amounts, time of administration, and $C_{(0)}$, CCS%, or AUEC%.

Oral Administration of Prednisone

Prednisone is a prodrug of prednisolone. Prednisolone is the pharmacologically active form of prednisone *in vivo*. Clinically, the dose of prednisone ranges from 5 to 200 mg. Prednisone is given once a day, or every other day. Therefore, two simulation scenarios were proposed, including single oral, and multiple oral dosing with either every 24 h, or every 48 h interval. In both situations, time of administration was selected within 24 h range at a 2 h interval, and dose amount was chosen based on therapeutic dose range of prednisone. For single oral dosing regimen, maximum concentrations of total prednisolone (C_{max}), time at maximum concentrations (T_{max}), CCS% and AUEC% were simulated. With multiple oral administrations, maximum concentrations (C_{max}), trough concentrations of total prednisolone (C_{trough}), CCS% and AUEC% at steady state were monitored and reported. All results were plotted in contour graphs.

RESULTS

Predictability of the Algorithm

Recently a new PK/PD approach was published to predict total prednisolone in plasma which adequately predicts literature PK data (18). We extended this approach and combined other literature information to predict PD of prednisolone, including cortisol suppression, and inhibition of lymphocytes trafficking in plasma (21,22) (Fig. 1). We developed an algorithm (Fig. 2) to predict PK/PD after prednisolone/prednisone administration. Appendix 1 describes how to use this algorithm in Excel® spreadsheet. In Fig. 3a,c, simulated cortisol and total lymphocyte trafficking baselines from the algorithm are plotted with observed data in literature (21–27). In Fig. 3b,d, cortisol suppression and inhibition of total lymphocyte trafficking profiles are compared with the data reported in actual clinical studies with

Fig. 3. Time-course of total cortisol concentrations **a** and **b** and total lymphocyte percentages of the count at 8:00AM **c** and **d** in plasma. **a** Cortisol baseline (black square ref (23), white circle ref (24), black circle ref (25), inverted black triangle ref (26), white triangle ref (22).); **b** Cortisol suppression (black diamond (16.4 mg IV PNL), dark gray diamond (49.2 mg IV PNL): ref (29), light gray diamond (29.7 mg IV PNL): ref (28).); **c** Total lymphocyte percentage baseline (white circle ref (21), black circle ref (23), white triangle ref (27).); **d** Inhibition of total lymphocyte trafficking (black square (37 mg PO PNL): ref (30) black triangle, gray triangle, light gray triangle (20 mg PO PN), and dark gray triangle (17.5 mg PO PN): ref (27), black circle (40 mg PO PNL), open circle (20 mg PO PNL), black diamond (20 mg PO PNL): ref (31).). Lines are simulated profiles using the algorithm. PO and IV represent oral and intravenous administrations. PNL and PN are abbreviations of prednisolone and prednisone

prednisolone/prednisone exposure (27–31). These results elucidated the validity of the PD prediction of this algorithm by visual inspection.

The ratios of the area under the cortisol concentration curve (AUC_{SR}) within 24 h of prednisone/prednisolone exposure have been reported in several studies (14,25,26,32). This parameter can be readily converted to CCS% ($CCS\% = 1 - AUC_{SR}$). Thus, several simulations were implemented to further illustrate the predictive power of this algorithm. Two studies with oral administration of prednisone were reported by Chakraborty *et al.* and Meno-Tetang *et al.* (25,32). The cortisol suppression simulated using this algorithm is close to

the reported data. Results are shown in Table I. Results were also extracted from two other clinical studies with intravenous administration of prednisolone (14,26), and the differences between the predicted CCS% and CCS% in literature are less than 10% (Table I).

Simulation of Intravenous Administration of Prednisolone

Figure 4 shows the results from the first simulation. Initial total prednisolone concentrations ($C_{(0)}$) are presented in Fig. 4a. CCS% and AUEC% were generated and plotted in Fig. 4b,c, respectively, with the oscillation pattern of CCS%

Table I. Comparison of Simulated Cumulative Cortisol Suppression (CCS) Results with Literature Reported Values

Drug	Dosing regimen	Dose amount (mg)	Reported CCS% ^a	Simulated CCS%	Difference %
Prednisone	Oral, single	15 ^b	51.00	54.15	3.15
		20 ^c	64.00	56.73	–7.27
Prednisolone	Intravenous, single	14.8 ^d	62.71	54.46	–8.25
		52.5 ^e	70.00	65.88	–4.12

^a Reported CCS% is calculated based on the area under curve of suppression ratios (AUC_{SR}) within 24 h from literature (mean values)
^b Reference (25)
^c Reference (32)
^d Reference (26)
^e Reference (14)

Fig. 4. Contour plots of initial total prednisolone concentrations ($C_{(0)}$) **a**, cumulative cortisol suppression (CCS%) **b**, and alteration of total lymphocyte trafficking (AUEC%) **c**, after single intravenous administration of prednisolone (the range of dose: 2–100 mg)

being the most pronounced. When prednisolone is administered around 6:00AM, $C_{(0)}$ is lowest. However, the lowest CCS% and the highest AUEC% occur when prednisolone is given between 9:00AM to 3:00PM, and the maximum CCS% and the minimum AUEC% are observed when the drug is given in the early morning between 2:00 AM and 4:00AM within various dose amounts.

In Fig. 5, results from the second simulation with small dose amounts (0.01–1 mg) are plotted. This simulation shows that when the dose amount is small, $C_{(0)}$ is time-dependent, and fluctuation of $C_{(0)}$ is obvious. Compared to the results in the first simulation, the lowest CCS% and the highest AUEC% are observed when the drug is given in the late afternoon, around 6:00PM. In addition, the possible stimulation of the lymphocyte trafficking, as indicated by the negative values of AUEC%, was identified within this dose range. The nadir of the effect of lymphocyte trafficking takes place when a dose (about 0.3 mg) is administered at approximately 4:00 AM.

Simulation of Oral Administration of Prednisone

Figure 6 shows the simulation results after a single oral dosing of prednisone. The effects of dosing time of prednisone on $C_{(0)}$, CCS%, and AUEC% seem to be similar to

those in Fig. 4, except for the larger dose range than that in the previous simulation. Time-dependency for the time of maximum total prednisolone concentrations ($T_{\max}$) was also evaluated (data not shown here), and it was found that $T_{\max}$ is around 1.3 h after dosing, and no differences or fluctuations of $T_{\max}$ are observed among various dosing amounts and dosing time.

In Fig. 7b, trough concentration (C_{trough}) in a multiple dosing regimen (once a day) is apparently time-dependent, and the minimum C_{trough} is observed when prednisone is taken at around 6:00AM. The patterns of $C_{\max}$, CCS%, and AUEC% in Fig. 7a,c, d are comparable to those after a single oral dosing in Fig. 6. Oral administration of prednisone every other day (48 h dosing interval) was also simulated, and similar results are obtained (plot not shown here).

DISCUSSION

Chronological biology and physiology, such as circadian patterns of major endocrine systems, lymphatic systems, and blood pressure, are the foundation of chronotherapy (1). Chronotherapy has been applied for the treatment of certain diseases, including hypertension, coronary heart disease, inflammation, and cancer (1–4). This strategy has provided

Fig. 5. Contour plots of initial total prednisolone concentrations ($C_{(0)}$) **a**, cumulative cortisol suppression (CCS%) **b**, and alteration of total lymphocyte trafficking (AUEC%) **c**, after single intravenous administration of prednisolone (the range of dose: 0.01–1 mg)

another method to improve the efficacy and safety or the risk-benefit values in the clinic. Ciclosporin and tacrolimus are two commonly used immunosuppressive drugs for the patients after organ transplantation. Several studies have found that pharmacokinetics of ciclosporin and tacrolimus is time-dependent. Compared to exposure after administration in the evening, higher exposure after morning dosing of ciclosporin or tacrolimus was observed, which has been attributed to the change of clearance (4). Statins, including simvastatin, lovastatin, pravastatin and rosuvastatin, which are popular drugs for lowering low-density lipoprotein cholesterol (LDL-C), have shown time-dependence of the effects from several clinical studies (33). All results have suggested that better lowering of LDL-C concentrations could be obtained after evening administration of statins compared to the morning dosing. The time-dependence of statin mediated lowering of LDL-C levels could result from the relatively short half life of statins (less than 5 h), as well as increased cholesterol production in the very early morning (circadian burst of cholesterol synthesis) (33). Recently, Glass-marmor *et al.* found that night-time treatment of methylprednisolone was preferred by patients with multiple sclerosis although morning administration is generally recommended for corticosteroid treatment (2). Thus, an under-

standing of time-dependency in the system, including PK and PD, is essential for chronotherapy.

Prednisolone has been extensively researched, and the involvement of circadian variation of cortisol release and nonlinear protein binding may lead to the time-dependency of the PK/PD of prednisolone. In this study, we integrated our PK/PD approach with other literature information to created an algorithm to predict both PK and PD, including cortisol suppression and inhibition of lymphocyte trafficking after prednisolone or prednisone administration (18,21,22). The validity of the PK/PD prediction was elucidated by visual inspection and comparison between simulated results and reported data in literature. These results ensured further simulation steps.

In simulations of both single and multiple dosing regimens, maximum total prednisolone concentrations were monitored (Figs. 4a, 5a, 6a, 7a). As expected, the maximum total prednisolone concentrations showed time-dependency because of the circadian rhythm of cortisol release, resulting in the oscillation of nonlinear protein binding of prednisolone; however, when the dose amount of exogenous corticosteroids increases, the impact of dosing time on total prednisolone concentrations diminishes due to the suppression of endogenous cortisol release, and the contribution of

Fig. 6. Contour plots of maximum total prednisolone concentrations ($C_{\max}$) **a**, cumulative cortisol suppression (CCS%) **b**, and alteration of total lymphocyte trafficking (AUEC%) **c**, after single oral administration of prednisone (the range of dose: 5–200 mg)

competitive nonlinear protein binding to the total prednisolone concentrations is reduced. In Fig. 7b, when oral dose is given once a day, C_{trough} concentrations are influenced by the time of administration of prednisone. This effect is due to the low concentration of prednisolone and less inhibition of cortisol release, which makes the impacts of cortisol circadian rhythm and competitive protein binding on C_{trough} significant. Interestingly, when $T_{\max}$ was assessed after a single oral administration, dosing amounts and time of administration did not show considerable effects on $T_{\max}$, which suggests that the absorption process of prednisone/prednisolone is neither dose- nor time- dependent. This result is also consistent with our PK model for free prednisolone and prednisone with first-order absorption processes (18).

When cumulative PD effects within 24 h, including CCS% and AUEC%, were plotted with the dosing amounts and time of administration as shown in Figs. 4b,c, 5b,c, 6b,c, and 7c,d, both CCS% and AUEC% exhibit dose- and time-dependency, and the oscillation pattern in CCS% plots is more pronounced than that in AUEC% plots. As described in Eq. 6, cortisol suppression is only dependent on the amount of exogenous corticosteroids present *in vivo*. However, alteration of lymphocyte trafficking in plasma is

controlled by both endogenous cortisol and exogenous corticosteroids as shown in Eq. 10 (21,22). Thus, the absolute active corticosteroid level in the system could be more, equal, or even less than baseline endogenous cortisol concentration since the body cannot distinguish between endogenous and exogenous corticosteroids. Therefore, the effect on lymphocyte trafficking may be inhibition or stimulation as indicated by either positive or negative values of AUEC%. Figure 5c illustrates the stimulation effect on lymphocyte trafficking when a small dose of prednisolone is given. However, this stimulation effect may not be evident in the clinic because of the low dose level. The simulated results also confirmed that the administration of corticosteroids in the morning is reasonable as indicated by the minimum cortisol suppression and maximum inhibition of total lymphocyte trafficking observed in this study.

Furthermore, it is also of great importance to consider the circadian patterns of the respective disease states. In RA patients, the clinical symptoms not only show a circadian rhythm pattern, but are almost orchestrated with the release of some cytokines and hormones in blood, including IL-6 and cortisol (34). Although there is a delay between the levels of symptoms (e.g. stiffness, and pain) and serum concentrations

Fig. 7. Contour plots of maximum total prednisolone concentrations ($C_{\max}$) **a**, trough total prednisolone concentrations (C_{trough}) **b**, cumulative cortisol suppression (CCS%) **c**, and alteration of total lymphocyte trafficking (AUEC%) **d**, at steady state after multiple oral administration of prednisone (QD, the range of dose: 5–200 mg)

of the cytokines or hormones, the worst disease-related symptom is also coupled to the highest levels of some cytokines or hormones in the morning (34). For the treatment of RA, it could be shown that timing of prednisolone administration might be critical in determining its effect on the diurnal rheumatoid inflammatory process. A total of 26 patients with rheumatoid arthritis were randomly divided into two equal groups and allocated to low doses of prednisolone at either 2:00 AM or 7:30 AM. Because of the diurnal variation in disease activity in RA, assessments of the two study groups were performed at 7:30 AM both at the start of the study (day 1) and after four doses of prednisolone (day 5). Administration of low doses of prednisolone (5 or 7.5 mg daily) at 2:00 AM had favorable effects on the duration of morning stiffness, joint pain, several disease indices and morning serum concentrations of IL-6. The other study group showed minor but significant effects on morning stiffness and circulating concentrations of IL-6 (8). Hence, administration of low doses of corticosteroids with a rather short biological half life seems to improve acute RA symptoms if it precedes the period of circadian flare in inflammatory activity, as defined by enhanced IL-6 synthesis. This data suggests that there is a circadian rhythm of the disease pattern with the optimum relieve of symptoms when prednisolone is adminis-

tered in the very early morning hours. In a recent study, Buttgerit *et al* applied a modified-release prednisone in RA patients, and a similar conclusion was drawn (35). Furthermore, the results from this study indicated that with a modified formulation in a timed release manner, the issue of patient compliance could be compromised and a small dose amount could be applied for better risk-benefit value.

Although the PK/PD simulations conducted in our study are not based on a stochastic approach and disease pattern, the approach is predictable and adequate. To our knowledge, this study is the first one to show the time-dependency of the PK/PD of prednisolone. Our results suggest that the current clinical use of prednisolone could be improved with a chronotherapy approach to find an optimal time of administration with higher efficacy and lower side effects.

CONCLUSIONS

We developed an interactive algorithm to predict the PK/PD of prednisolone after prednisolone/prednisone administration. This algorithm was evaluated visually for the predictability. Several simulations were performed to assess the impact of dosing time on the PK/PD of prednisolone. The results revealed time-dependency of the PK/PD of prednisolone,

and suggested that it is necessary to consider the application of chronotherapy in order to achieve better clinical outcomes with fewer side effects of prednisolone. This PK/PD simulation approach could provide a valuable tool to evaluate and predict dosing time effect, and direct clinical use of prednisolone.

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APPENDIX 1

Description of the algorithm interface in Excel® spreadsheet. Two files were created in Excel® as 97-2003 worksheets: 1-Intravenous_Admin for intravenous administration of prednisone or prednisolone; 2-Oral_Admin for oral administration of prednisone or prednisolone. Interactive algorithm is shown in Figure 2.

1. Input Section

Drug Name: pull-down menu (Prednisone or Prednisolone)

Dose (mg): dose amount (single dose) or each dose amount (multiple doses)

Time of Admin: time of dosing (24hr scale (0 to 24), not clock scale) (e.g. 8.5 for 8:30 AM, 20.5 for 8:30 PM)

Dose Regimen: pull-down menu (Single or Multiple)

Tau: dosing interval for multiple doses (only apply for the multiple dose regimen)

2. Output Section

CCS% (24hr): cortisol suppression within 24 hr compared with baseline

ΔAUEC% (24hr): alteration of total lymphocyte trafficking within 24 hr compared with baseline

Pharmacokinetic Profiles: free and total prednisone, free and total prednisolone concentration-time profiles

Pharmacodynamic Profiles: plasma cortisol concentration and alteration of plasma total lymphocyte trafficking (%) time profiles at baseline and after treatment of prednisone or prednisolone

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