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Fifth-Generation Model for Corticosteroid Pharmacodynamics: Application to Steady-State Receptor Down-Regulation and Enzyme Induction Patterns during Seven-Day Continuous Infusion of Methylprednisolone in Rats

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Abstract

A fifth-generation model for receptor/gene-mediated corticosteroid effects was proposed based on results from a 50 mg/kg IV bolus dose of methylprednisolone (MPL) in male adrenalectomized rats, and confirmed using data from other acute dosage regimens. Steady-state equations for receptor down-regulation and tyrosine aminotransferase (TAT) enzyme induction patterns were derived. Five groups of male Wistar rats ($n=5/\text{group}$) were subcutaneously implanted with Alzet mini-pumps primed to release saline or 0.05, 0.1, 0.2, and 0.3 mg/kg/hr of MPL for 7 days. Rats were sacrificed at the end of the infusion. Plasma MPL concentrations, blood lymphocyte counts, and hepatic cytosolic free receptor density, receptor mRNA, TAT mRNA, and TAT enzyme levels were quantitated. The pronounced steroid effects were evidenced by marked losses in body weights and changes in organ weights. All four treatments caused a dose-dependent reduction in hepatic receptor levels, which correlated with the induction of TAT mRNA and TAT enzyme levels. The 7 day receptor mRNA and free receptor density correlated well with the model predicted steady-state levels. However, the extent of enzyme induction was markedly higher than that predicted by the model suggesting that the usual receptor/gene-mediated effects observed upon single/intermittent dosing of MPL may be countered by alterations in other aspects of the system. A mean IC_{50} of 6.1 ng/mL was estimated for the immunosuppressive effects of methylprednisolone on blood lymphocytes. The extent and duration of steroid exposure play a critical role in mediating steroid effects and advanced PK/PD models provide unique insights into controlling factors.

Keywords

pharmacodynamics; pharmacogenomics; methylprednisolone; tyrosine amino-transferase

INTRODUCTION

Corticosteroids are an important class of drugs used for the treatment of immunological conditions associated with various diseases such as rheumatoid arthritis (1), lupus erythematosus (2), bronchial asthma (3), and renal diseases as well as for organ transplantation. Many of these diseases are chronic in nature and therefore necessitate long-term steroid therapy.

Steroid responses can be classified as rapid effects (cell trafficking, cortisol suppression) or delayed effects (receptor/gene-mediated enzyme induction/repression) depending on the time course of the response (4). The enzymes that are induced or repressed via pharmacogenomic processes include both metabolic and immunosuppressive genes. The metabolic effects are generally responsible for many of the complications and undesirable effects associated with long-term steroid therapy (5). It is therefore essential to understand the regulation of expression of these genes and the rate and extent to which they are affected by steroid treatment. Further, it is important to determine whether there are any alterations in gene expression during chronic therapy. Most effects of steroids are mediated via binding to the cytosolic glucocorticoid receptor. Hence, changes in receptor dynamics or auto-regulation during prolonged treatment can lead to altered responses.

Development of mechanistic PK/PD models for corticosteroid effects is vital to enable a quantitative understanding of the molecular events and mechanisms involved in mediating steroid effects. A series of pharmacodynamic models have been proposed to explain the time course of receptor dynamics and enzyme induction upon single IV bolus dosing of steroids (6–9). The most current “fourth-generation” model (8) satisfactorily captures the data patterns of steroid gene-mediated responses upon a 50 mg/kg IV bolus dose of methylprednisolone in adrenalectomized rats. However, the model had to be extended and a new set of parameters was obtained to describe the data from the subsequent double-dose study. The primary aim of this report is to present an improved mechanistic model describing the gene-mediated effects of corticosteroids using data from several doses and dosage regimens. A major improvement of our present fifth-generation model over the earlier one is that it is structurally more mechanistic allowing it to adequately characterize data from several studies using one single set of parameters. Further, we used the model to make predictions for steady-state receptor dynamics and enzyme induction conditions which were then experimentally confirmed. We also examined several indicators of toxicity and efficacy of steroid treatment such as net changes in body weights, weights of different organs, and lymphocyte trafficking patterns in blood during 7 days of infusion in adrenalectomized rats.

Tyrosine aminotransferase (TAT) enzyme is the rate-limiting and regulatory factor controlling tyrosine metabolism (10). This is an ammonia-detoxifying enzyme that indirectly controls glucose production in the liver by providing a gluconeogenic substrate (11). It is one of the most well-studied and well-characterized enzymes which reflects a prototype response in terms of gene-mediated steroid induction. Moreover, unlike certain other steroid-induced enzymes such as glutamine synthetase whose levels are thought to be dependent on concentrations of the product synthesized (12,13), TAT activity is not

influenced by tyrosine. These reasons made TAT an ideal marker for studying the acute and long-term gene-mediated effects of the steroid. Steady-state responses upon administration of four different infusion regimens of methylprednisolone were measured. Preliminary studies indicated that a seven-day infusion period is sufficient to allow steady-state in the responses to be attained. A subcutaneous (SC) infusion regimen was chosen since it acts as a means of continuous controlled delivery of the drug. It is the simplest mode of administration to ensure steady-state levels of the steroid in circulation, partly approximating clinical situations where daily steroid is administered over months.

MATERIALS AND METHODS

Animals

Male adrenalectomized (ADX) Wistar rats (377 ± 35 g) were purchased from Harlan-Sprague Dawley Inc. (Indianapolis, IN). All animals were housed in a 12 hr light/dark cycle and acclimatized in a constant temperature environment of 22°C for at least one week. Rats had free access to 0.9% NaCl drinking water. One day prior to the study, rats underwent right external jugular vein cannulation under ketamine/xylazine anesthesia. Cannula patency was maintained with sterile 0.9% NaCl solution. Rats had free access to a preweighed amount (250 g) of rat chow. This research adheres to "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985) and was approved by the Institutional Animal Care and Use Committee of our university.

Experimental

Rats were divided into five groups. The four treatment groups ($n=5$ /group) were administered 7-day infusions of 0.05, 0.1, 0.2, and 0.3 mg/kg/hr of methylprednisolone sodium succinate (Solu-Medrol, The Upjohn Company, Kalamazoo, MI) reconstituted with supplied diluent. Alzet osmotic mini-pumps (Model 2001, Alza, Palo Alto, CA) with a flow rate of $1 \mu\text{l/hr}$ were subcutaneously implanted in order to obtain a zero-order delivery of the drug. The pump had a drug reservoir volume of $200 \mu\text{l}$. Therefore, to obtain release rates of 0.05, 0.1, 0.2 and 0.3 mg/kg/hr, the equivalent concentrations of solutions placed in the pump were 10, 20, 40 and 60 mg/kg. For each rat, the concentration of the pump solution was prepared based on the predose body weight of the rat. The pumps were equilibrated for at least 4 hr at 37°C in saline prior to implantation in order to ensure a constant zero-order release rate. The control group ($n=7$) was implanted with saline filled pumps.

Blood samples ($700 \mu\text{l}$) were withdrawn from each rat (via the cannula) before implanting the pump for predose lymphocyte counts as well as daily for plasma methylprednisolone concentrations. The body weight of each rat was recorded on a daily basis. Rats were sacrificed by aortic exsanguination under ketamine/xylazine anesthesia at the end of seven days. Various organs including the liver, spleen, thymus, gastrocnemius muscle, lungs, heart, and kidney were excised and weighed. The liver was rapidly excised and 1 g placed in ice-cold buffer solution for TAT enzyme activity measurements while the remainder was flash frozen in liquid nitrogen. The tissues were stored at -80°C until analysis was performed. A $100 \mu\text{l}$ portion of the blood drained at sacrifice from the abdominal aortic artery was immediately used to determine blood lymphocyte counts while the remainder was

collected into a heparinized syringe and centrifuged. Plasma was harvested and frozen at -20°C . Food intake for the entire seven-day period was measured by the difference between the preweighed amount of rat chow provided at the start of the experiment and that remaining in the cage at sacrifice.

Assays

Plasma methylprednisolone concentrations were determined by a normal phase high-performance liquid chromatography (HPLC) method as described earlier (8,14). The limit of quantitation was 10 ng/mL. Hepatic cytosolic glucocorticoid receptor concentrations were determined using a previously established radiolabeled ligand binding assay (8,15). The cytosolic receptor density ($B_{\max}$) was estimated by solving the following equations simultaneously:

$$D_T = D_{NS} + \frac{B_{\max} \cdot D_f}{K_D + D_f} \quad \text{and} \quad D_{NS} = K \cdot D_f \quad (1,2)$$

where K_D is the equilibrium dissociation constant for specific drug-receptor binding and K is the linear non-specific binding constant. The mRNA for the receptor and TAT was assayed using quantitative Northern hybridization (16,17). The TAT activity in the hepatic cytosol was determined by the Diamondstone colorimetric method (18). Protein content in the tissues was measured using the Lowry method (19) using bovine serum albumin standards. The results for receptor density and TAT activity were normalized by the protein content in the samples. Blood lymphocyte counts were measured using the automated CELL-DYN[®] 1700 system (Abbott Laboratories).

Data for the acute dosing studies were obtained from results published previously by our laboratory (8,9). These included two studies where single intravenous bolus doses of 50 mg/kg, 10 mg/kg and double doses of 50 mg/kg at 0 and 24 hr were administered to male adrenalectomized Wistar rats that had undergone right jugular vein cannulation. Pharmacodynamic markers for the gene-mediated effects included receptor mRNA, free receptor density, TAT mRNA and TAT activity in rat liver cytosol.

THEORETICAL

Pharmacokinetics

For the data analysis from the single and double dose intravenous bolus studies, the pharmacokinetic equations with the parameters as reported earlier in the corresponding papers (8,9) were utilized to drive the dynamics (Table I). The biexponential kinetics of methylprednisolone after the 50 mg/kg dose was described by:

$$C_{MPL} = C_1 \cdot e^{-\lambda_1 \cdot t} + C_2 \cdot e^{-\lambda_2 \cdot t} \quad (3)$$

where C_i and λ_i are the coefficients for the intercepts and slopes and C_{MPL} is the plasma concentration of methylprednisolone. A two-compartment model was used to describe the kinetics of methylprednisolone following the 10 mg/kg single and 50 mg/kg double doses. The differential equations for the model include:

$$\frac{dA_p}{dt} = k_{21} \cdot A_t - k_{12} \cdot A_p - \left(\frac{CL}{V_p} \right) \cdot A_p \quad (4)$$

$$\frac{dA_t}{dt} = k_{12} \cdot A_p - k_{21} \cdot A_t \quad \text{and} \quad A_p = C_p \cdot V_p \quad (5,6)$$

where A_p and A_t are the amounts of drug in the plasma and tissue compartments, C_p is the methylprednisolone concentration in the plasma, k_0 is the zero-order rate constant for drug input into the plasma, CL is the clearance, V_p is the plasma volume of distribution and k_{12} and k_{21} are the intercompartmental distribution rate constants.

Pharmacodynamics

Mechanism of Steroid Action—As depicted in Fig. 1, unbound methylprednisolone in the blood being highly lipophilic freely diffuses into the cytoplasm of the liver parenchymal cell. Binding of the steroid to the cytosolic glucocorticoid receptor causes activation of the receptor and this is accompanied by release of heat shock proteins, hyperphosphorylation and conformational changes in the receptor. The activated drug-receptor complex rapidly translocates into the nucleus where it binds as a dimer to the glucocorticoid responsive element (GRE) in the DNA. This leads to the enhanced or repressed expression of numerous genes. In our case, the TAT gene studied is enhanced by steroid treatment. Binding of the activated receptor to the GRE enhances the rate of transcription of the TAT mRNA which then is translated to TAT enzyme in the cytosol. At the same time, steroids are known to cause homologous down-regulation of their own receptor (20–22). Specifically, binding of the activated steroid-receptor complex to the GRE results in reduced levels of receptor mRNA. This further leads to decrease in the free receptor density in the cytosol.

Fifth-generation Model for Corticosteroid Receptor/Gene-Mediated Effects—

The fifth-generation pharmacodynamic model for corticosteroid receptor/gene-mediated effects is shown in Fig. 2. The differential equations for the various components of the model controlling receptor regulation included

$$\frac{dR_m}{dt} = k_{\text{syn-} R_m} \cdot \left(1 - \frac{DR(N)}{IC_{50-} R_m + DR(N)} \right) - k_{\text{deg-} R_m} \cdot R_m \quad (7)$$

$$\frac{dR}{dt} = k_{\text{syn-} R} \cdot R_m + R_f \cdot k_{re} \cdot DR(N) - k_{on} \cdot D \cdot R - k_{\text{dgr-} R} \cdot R \quad (8)$$

$$\frac{dDR}{dt} = k_{on} \cdot D \cdot R - k_T \cdot DR \quad (9)$$

$$\frac{dDR(N)}{dt} = k_T \cdot DR - k_{re} \cdot DR(N) \quad (10)$$

where symbols represent the mRNA for the receptor (R_m), the free cytosolic receptor density (R), cytosolic drug-receptor complex (DR) and nuclear activated drug-receptor complex ($DR(N)$) as well as the first-order rates of synthesis (k_{syn}) and degradation (k_{dgr}) of the response. Also, IC_{50_Rm} is the concentration of $DR(N)$ at which the synthesis rate of receptor mRNA for the receptor drops to 50% of its baseline value, k_{on} is a second-order rate constant for drug-receptor binding, and k_T and k_{re} are the first-order rates of receptor translocation to the nucleus and recycling back from the nucleus to the cytosol. Further, R_f is the fraction of drug recycled and D corresponds to the molar drug concentrations as governed by the kinetics of the methylprednisolone in plasma.

The baselines were defined using the following equations:

$$k_{dgr_Rm} = \frac{k_{syn_Rm}}{R_{m0}} \quad (11)$$

$$k_{syn_R} = \left(\frac{R_0}{R_{m0}} \right) \cdot k_{dgr_R} \quad (12)$$

where R_{m0} and R_0 , the baseline values of mRNA for receptor and free cytosolic receptor density, were fixed based on the mean values of the control animals. Parameters reported in the fourth-generation model (8) were used for simulations of the receptor dynamics for the different dosage regimens.

The enzyme dynamics were fitted to the following equations:

$$\frac{dTAT_m}{dt} = k_{syn_tm} \cdot (1 + S \cdot DR(N)) - k_{dgr_tm} \cdot TAT_m \quad (13)$$

$$\frac{dTAT}{dt} = EF \cdot (TAT_m)^\gamma - k_{dgr_t} \cdot TAT \quad (14)$$

where TAT_m and TAT are the mRNA for TAT and the TAT enzyme, S is the linear constant for efficiency of TAT gene induction by $DR(N)$, EF is the efficiency of translation of TAT mRNA to TAT enzyme, γ is an amplification exponent for the translation, k_{syn_tm} is the zero-order rate of TAT mRNA synthesis, and k_{dgr_tm} and k_{dgr_t} represent the first-order rates of degradation of TAT mRNA and TAT.

The baseline equations included:

$$k_{syn_tm} = k_{dgr_tm} \cdot TAT_{m0} \quad (15)$$

$$EF = \left(\frac{TAT_0}{(TAT_{m0})^\gamma} \right) \cdot k_{dgr_t} \quad (16)$$

The equations were fitted simultaneously to the TAT dynamic data from the 50 mg/kg dose study. The TAT_{m0} and TAT_0 are the baseline levels of TAT mRNA and TAT and were

estimated from the fitting. The parameters estimated were used to perform simulations for the other dosage regimens in order to validate the model. The baselines for these simulations were obtained from the mean data from the control animals, as listed in Table I.

The fifth-generation model comprises of several modifications made to the fourth-generation model (8) including the following:

1. A steroid-independent zero-order synthesis of TAT mRNA was included. It was assumed that mRNA for TAT degrades with a first-order rate constant, $k_{\text{deg_tm}}$.
2. The hypothetical transcription compartment TC was eliminated.
3. The enhancement of TAT mRNA by steroid was modeled as an indirect stimulation of the zero-order production of TAT mRNA. A linear stimulation function S was used to indicate the efficiency of gene induction.
4. The interaction of the activated receptor with the DNA was assumed to occur in the $DR(N)$ which was consequently used to drive the down-regulation of the receptor mRNA. Down-regulation of receptor mRNA could occur as a result of inhibition of synthesis $k_{\text{syn_Rm}}$ (submodel A) and/or stimulation of degradation $k_{\text{dgr_Rm}}$ (submodel B). There is *in vitro* experimental evidence in the literature supporting both mechanisms (23,24). We preferred submodel A based on the likelihood ratio test and the fact that the estimated parameter values using this submodel were more relevant physiologically.
5. The degradation rate constant $k_{\text{dgr_Rm}}$ was expressed as a function of $k_{\text{syn_Rm}}$ and the baseline receptor mRNA levels. Thus, differences in baseline receptor mRNA levels among the different studies were accounted for by a corresponding change in the receptor mRNA degradation rate.

Steady-State Equations—In order to obtain the expected receptor and TAT dynamics at the end of the different infusions, steady-state equations were derived for our model:

$$R_{ss} = \frac{k_{\text{syn_R}} \cdot R_{m,ss} + (R_f \cdot k_{re}) \cdot DR(N)_{ss}}{k_{\text{on}} \cdot D_{ss} + k_{\text{dgr_R}}} \quad (17)$$

$$DR_{ss} = \frac{k_{\text{on}} \cdot D_{ss} \cdot R_{ss}}{k_T} \quad (18)$$

$$DR(N)_{ss} = \frac{k_T \cdot DR_{ss}}{k_{re}} \quad (19)$$

$$R_{m,ss} = \frac{k_{\text{syn_Rm}} \cdot \left(1 - \frac{DR(N)_{ss}}{IC_{50_Rm} + DR(N)_{ss}}\right)}{k_{\text{dgr_Rm}}} \quad (20)$$

$$TAT_{m,ss} = \frac{k_{\text{syn- tm}} \cdot (1 + S \cdot DR(N)_{ss})}{k_{\text{dgr- tm}}} \quad (21)$$

$$TAT_{ss} = \frac{EF \cdot (TAT_{m,ss})^\gamma}{k_{\text{dgr- t}}} \quad (22)$$

The above equations along with the baseline equations were simultaneously used to obtain steady-state responses for the various gene-mediated pharmacodynamic measures for the four dosing groups. The molar steady-state drug concentration for the infusion period for each dose is represented by D_{ss} . Simulations were performed to define the effects of alterations in the drug-receptor binding (k_{on}), receptor recycling (k_{re}) rates as well as the transcription efficiency (S) and TAT mRNA amplification factor (γ) on steady-state responses as a function of the steady-state molar plasma drug concentrations.

Lymphocyte Trafficking—The cell-trafficking model used to quantitate steady-state dynamics yields:

$$R_{ss} = R_0 \cdot \left(1 - \frac{I_{\max} \cdot C_{ss}}{IC_{50} + C_{ss}} \right) \quad (23)$$

Hence,

$$IC_{50} = C_{ss} \cdot \left(\frac{R_0 \cdot I_{\max}}{R_0 - R_{ss}} - 1 \right) \quad (24)$$

where R_{ss} represents the steady-state lymphocyte counts, R_0 is the baseline which was fixed to a 100%, $I_{\max}$ is the maximum possible suppression of lymphocytes by MPL, IC_{50} is the concentration of drug needed to produce half-maximal suppression of lymphocyte entry into blood, and C_{ss} is the steady-state MPL concentration. It was assumed that when given in sufficiently large doses, MPL can completely reduce the lymphocytes in blood to zero and hence the $I_{\max}$ was fixed to 1. Under this special condition when $I_{\max}$ is 1, equation 24 becomes:

$$IC_{50} = C_{ss} \cdot \left(\frac{R_{ss}}{R_0 - R_{ss}} \right) \quad (25)$$

The 7-day lymphocyte counts (R_{ss}) were expressed as a percent of the pre-dose value (defined as 100%) for each animal. The mean percent predose lymphocyte counts were used for the calculation of the IC_{50} at each of the infusion regimens.

Data Analysis—The ADAPT II program (25) with the maximum likelihood method was used for all fittings and simulations. The underlying assumption is that errors from the observed and predicted responses are normally distributed. The variance model specified

was: $V(\sigma, \theta, t_i) = \sigma_1^2 \cdot Y(\theta, t_i)^{\sigma_2}$, where V is the variance of the i th point and θ represents the vector of parameters. The variance parameters σ_1 and σ_2 were fitted. Statistical analysis to detect significant differences between groups was performed using ANOVA with the Newman–Keuls post-hoc test (α set to 0.05). Comparisons between each treated and control group was performed using the unpaired Student t -test.

RESULTS

Pharmacokinetics

The average plasma methylprednisolone concentrations over the study period after the four infusion regimens are indicated in Table II. Steady-state is expected to be achieved at the first measured time point because of the short half-life of the drug (~30 min). The clearance (Infusion rate/ C_{ss}) ranged between 5 and 6 L/hr/kg in the different groups.

Pharmacodynamics

Food intakes for the treatment groups were not significantly different from those for the control group and the mean consumption was 36.6 % of the body weights per week. Control animals had essentially constant body weights throughout the study period. The four treated groups showed a dose-dependent reduction in body weights with time. Animals subject to the lowest and highest infusion rates had lost 10% and 25% of their body weights by the end of the study. All treated rats had 7-day liver weights higher than predose. The thymus showed the maximum involution (mean 22.3%) with the spleen, muscle, and lungs following. The kidney and heart weights remained constant. The present regimens appear to provide a paradigm for severe chronic adverse effects of corticosteroids.

Gene-Mediated Enzyme Induction

Figure 3 shows the fittings of our fifth-generation model to the data from the single 50 mg/kg IV bolus doses and Table I lists the parameters. Simulations were also done for the 10 mg/kg dose as shown in the figure. Down-regulation of the receptor mRNA with a minimum around 10 hr was observed. The mRNA returned close to baseline by 48 hr. The free cytosolic receptor levels fell immediately to zero and they returned to baseline by 72 hr in two phases. The first phase up to 8 hr was captured by rapid receptor recycling from the nucleus to the cytosol while the later slow phase was accounted for by translation from the newly transcribed receptor mRNA. The TAT mRNA and TAT profiles showed a slow rise peaking around 5 and 7 hr and returning back to baseline by 18 hr. The model seemed to capture the data patterns well. Further, the model was used to perform simulations to predict the expected time course of dynamic measures upon two doses of 50 mg/kg with doses given at 0 and 24 hr. As shown in Fig. 4, the model could adequately predict the tolerance seen in the TAT dynamics when the second dose was given. At the time the second dose was given, the receptors had not returned to baseline and there were fewer free receptors available for binding to the steroid at 24 hr which resulted in a lower extent of TAT induction. Since one set of parameters could adequately capture the receptor and TAT dynamics after a variety of dosage regimens, the present model seems to satisfactorily represent the gene-mediated events involved and affected by MPL.

Figure 5 shows the 7-day steady-state receptor mRNA and free receptor densities for the different groups in the infusion study. There were no significant differences in the observed receptor mRNA among the different groups. Free receptor density in liver cytosol on day 7 decreased as a function of infusion rate from 267 fmole/mg protein in the control group to mean values of 143.2, 53.1, 33.0, and 26.5 fmole/mg protein in the 0.05, 0.1, 0.2, and 0.3 mg/kg/hr dosing groups. The TAT mRNA and TAT activity at the end of the 7-day infusion are also shown in Fig. 5. No significant differences were found in the observed TAT mRNA among the groups. The TAT enzyme activity on day 7 in the liver increased as a function of infusion rate. Control rats showed a baseline TAT activity of 0.07 which rose to 0.08, 0.25, 0.27, and 0.47 in the treated groups in the order of increasing infusion rate. The expected 7-day measures of the receptor and TAT dynamics calculated using the steady-state equations are superimposed on the observed values. There was an excellent agreement between the observed and the model-predicted receptor mRNA and free cytosolic receptor density at day 7 for the three highest infusion regimens. The lowest infusion regimen showed 1.7-fold higher steady-state receptor mRNA levels and this was reflected in the corresponding higher free receptor levels in this group. The steady-state plasma methylprednisolone concentrations used to predict the dynamic steady-state responses for the lowest infusion rate were approximated as the limit of quantitation. One explanation could be that the actual mean concentrations were lower than this limit causing the observed down-regulation in the receptor dynamics to be overpredicted. The TAT mRNA profiles for the 0.1 and 0.3 mg/kg/hr doses seemed to be predicted well by the steady-state equations while the other two doses were overestimated. In any case, the observed data for all the treated groups were not significantly different from each other and from the control indicating that tolerance had developed over the one-week dosing period. Our steady-state predictions successfully captured this phenomenon. As a consequence, our model also accounts for tolerance in the steady-state TAT levels. Surprisingly, the observed 7-day TAT levels were consistently higher than expected based on extrapolation from acute dosing. In summary, the steady-state equations could well predict the receptor dynamics upon 7-day infusion of methylprednisolone. However, there was a discrepancy in the observed and expected TAT levels indicating some decoupling between receptor and TAT dynamics during 7-day MPL dosing.

Simulations

Figure 6 shows the steady-state receptor and enzyme responses predicted by our model over a range of MPL concentrations from 0 to 150 nM. As the concentrations increase, the receptor mRNA and free receptor density continue to decrease. For practical purposes, these levels can be assumed to stabilize at MPL concentrations around 100 nM. The TAT mRNA and TAT activity, on the other hand, reach close to their maximum limits at 50 nM of MPL. The effect of k_{on} , k_{re} , S and γ on the rate and extent of generation of the responses was evaluated.

A 10-fold increase in k_{on} resulted in a steeper drop in the receptor levels presumably because of a stronger receptor binding affinity and faster translocation to the nucleus. The receptor mRNA, TAT mRNA, and TAT levels reach their limits at MPL concentrations as low as 10 nM. Higher drug concentrations are associated with receptor mRNA and free

receptor levels that are lower than those if the k_{on} was 10-fold lower. However, the maximum TAT mRNA and TAT responses attainable do not differ substantially with a change in k_{on} . The extent of enzyme induction is MPL concentration dependent up to 50 nM after which they reach an asymptote irrespective of the k_{on} . A 10-fold decrease in the receptor-recycling rate led to a faster drop in the free receptor density, although the rate was slower compared to when the k_{on} was increased 10-fold. On the other hand, the receptor mRNA was down-regulated to a greater extent and the nadir was associated with MPL concentration around 30 nM. A slower return rate of the receptor can be assumed to be associated with higher levels of drug-receptor complex in the nucleus. This would result in a dual effect: a greater transcriptional down-regulation of the receptor mRNA levels as well as an increased transcription rate of the TAT gene. Our simulations confirm this since the receptor mRNA was maximally down-regulated and the TAT mRNA and TAT levels were dramatically increased. The concentrations at which the limits in the responses were reached did not seem to be dependent on the recycling rate. Thus k_{re} seems to control the maximal responses attainable whereas a change in k_{on} alters the MPL concentrations required to achieve maximal responses in TAT dynamics.

A two-fold increase in the linear transcription constant S simply amplified the TAT mRNA consequently also causing TAT activity to be higher. The final TAT activity was 2-fold higher at MPL concentrations above 50 nM. A change in the γ modifies the baseline relation between TAT mRNA and TAT. Doubling the γ caused an amplification in the translation of TAT mRNA and TAT leading to higher TAT levels. Any change in the degradation rates of TAT mRNA or TAT did not alter the curves since the production rates were automatically adjusted to give the same steady-state baseline response.

Lymphocyte Trafficking

As shown in Fig. 7, the percent predose 7-day lymphocyte counts for all treatment groups was significantly lower than that of the control. Table II lists the IC_{50} values obtained. The IC_{50} ranged from 4 to 11 ng/mL with a mean IC_{50} of 6.1 ng/mL which confirms the high immunosuppressive potency of methylprednisolone. The jointly calculated value across all doses was 7.52 ng/mL.

DISCUSSION

In this report, we have presented and confirmed an improved receptor/gene-mediated model for corticosteroid effects. The model is an extension of our previous work (8). Several modifications were made to the fourth-generation model. The ADX control animals do not have any measurable steroid in circulation. In spite of that, these rats produce baseline levels of TAT mRNA and TAT. This implies that a steroid-independent pathway of production of TAT mRNA governs the baseline response and this was modeled by introducing a zero-order production k_{syn_tm} for the message levels of TAT. It is well established in hepatoma cells (26), in primary hepatocytes (27) as well as in rat liver *in vivo* (28,29) that the TAT gene is transcriptionally controlled not just by glucocorticoids alone, but also by cAMP. It is possible that basal cAMP levels in the liver parenchymal cells are responsible for the baseline TAT levels or that the expression is never turned off, while steroids may act as

enhancers of the TAT gene. The enhancement of TAT gene transcription was modeled with a linear stimulation function, S , that indirectly augments the zero-order synthesis of TAT mRNA. The delayed peaks for TAT mRNA and TAT were captured in the earlier model using an empirical transcription compartment TC. However, it is more likely that the events post receptor-GRE binding are responsible for these delays. Hence, the TC compartment was eliminated and an indirect stimulation of the gene by the activated drug-receptor complex in the nucleus, $DR(N)$, was included. Since the receptor-DNA interaction was assumed to occur in $DR(N)$, it was appropriate to use this compartment as the driving force for the inhibition of receptor mRNA synthesis. The TAT enzyme levels solely depend on the translation of TAT mRNA and therefore, we related the baseline TAT levels to the baseline TAT mRNA using a first-order conversion rate. An amplification factor γ on the TAT mRNA to TAT translation was necessary in order to obtain a comprehensive model and parameters capable of describing the dynamics from all IV bolus dosage regimens. The exact relationship defining the translation of mRNA to enzyme is unknown. A first-order conversion EF with an amplification exponent seemed to best characterize all the data for TAT mRNA and TAT and hence was chosen. Moreover, the estimated degradation rate of TAT gave a half-life of 1 hr, which is close to experimental literature estimates of 1.5 hr (30).

Our simulations demonstrate the non-linear nature of the steady-state concentration-response relationship. The k_{on} can be assumed to be equivalent to the drug-receptor association rate while the k_{re} might reflect the drug-receptor dissociation rate. Steroids with different structures show different equilibrium dissociation constants (K_d), which is mainly due to different rates of dissociation (k_{off}) from the receptor. For example, dexamethasone and triamcinolone acetonide have K_d values three and eight times that of MPL in human lung tissue (31). Our simulations show that a decrease in the dissociation rate can markedly increase the maximal responses that can possibly be achieved at steady-state, which confirms that the degree of receptor affinity is reflective of the efficacy of various glucocorticoids on a cellular level. The MPL concentrations up to 50 nM were associated with increasing responses. However, steady-state concentrations above 50 nM did not differ in the extent of gene induction indicating that MPL dosage regimens delivering steady-state concentrations above 50 nM are not optimal. The optimal steady-state concentrations required for drugs with lower dissociation rate constants would be lower. The concentrations of MPL in our study ranged from 27 to 132 nM.

It is reported in the literature that baseline levels of the glucocorticoid receptor and TAT enzyme as well as its mRNA undergo oscillatory changes with a general decline in old age (11,32). The rats in the different studies were of various body weights and age ranges with considerable variation in the baseline responses. An alteration in either the degradation and/or synthesis rate of receptor mRNA can account for these different baselines among the different studies. It might be assumed that the rate of degradation remains constant while the synthesis rate changes as a function of baseline. However, for the receptor mRNA, simulations using our model indicated that a change in the degradation rate of receptor mRNA in the various age groups best characterized the data.

As shown in Fig. 3, the rate and extent of TAT mRNA and TAT expression could be completely accounted for by the kinetics of receptor occupancy. In the subsequent double-dose study, the effect of two consecutive doses of MPL at an interval of 24 hr on the same dynamic responses was investigated (9). Down-regulation of receptor led to a tolerance effect and the magnitude of TAT induction was lower after the second dose. Our present model could predict the dose-dependence and tolerance for these receptor/gene-mediated steroid effects upon acute dosing.

In the present study, we have examined the regulation of glucocorticoid receptor and TAT dynamics at the end of a 7-day infusion of methylprednisolone in male *ADX* Wistar rats. Silva *et al.* reported that maximal down-regulation of the receptor protein and mRNA occurs in HeLa S3 cells within 2 weeks of dexamethasone treatment and is completely reversible upon steroid removal (33). Similarly, we noted a marked down-regulation of the receptor mRNA and free cytosolic receptor densities in our *in vivo* study. The availability of a pharmacodynamic model allowed us to project steady-state conditions and make comparisons between the observed and model-predicted dynamics in order to determine if there were any changes in the receptor-regulation and/or signal transduction during continuous dosing. Steady-state levels predicted by the model coincided well with the 7-day receptor mRNA and free receptor density profiles. However, there was a marked discrepancy between the observed and predicted TAT enzyme activity indicative of some decoupling between receptor and TAT dynamics during chronic treatment. This leads us to believe that these exaggerated effects seen upon long-term dosing may be due to some modifications in post-receptor events. Cell culture studies have also suggested dissociation between agonist-induced decrease in receptor content and the steroid potency upon chronic exposure (34). This further leads to the question whether there are changes in signal transduction pathways following the initiation of gene transcription that are caused specifically as a result of chronic continuous dosing. Studies uncovering the entire temporal profile of receptor/gene events upon long-term dosing will provide insights into the processes that might be altered, and help us modify our model if necessary for accurate predictions of steroid dynamics upon chronic dosing.

It is well established that along with steroid pharmacokinetics, one of the major factors governing the extent of gene induction is the availability of free receptors and receptor auto-regulation (4). Our infusion studies indicate that this holds true upon longer-term dosing as well. The extent of induction and tolerance of the TAT gene was dependent on the free receptor density and could be extrapolated using parameters from acute dosing conditions. Daily cortisol administration of 50 mg/kg to normal rats for 15–22 days was shown to be associated with a 5-fold increase in TAT activity within 3 days followed by a 40% decrease at 7–10 days. A similar time course was observed with other gluconeogenic enzymes including glucose-6-phosphatase (7%) and fructose-1,6-diphosphatase (8%) in rat liver (35). We observed a 1- to 7-fold increase in the steady-state TAT activity for the different infusion groups. The highest infusion rate group received a total MPL dose of 50.4 mg/kg over the 7 days, which was associated with a 12-fold increase in peak TAT activity in the 50 mg/kg acute dosing study. This indicates that some tolerance in the final enzyme effects can be expected based on the dose, length, and mode of administration, although the extent of

tolerance seems to differ from that projected by our fifth-generation model. In order to better appreciate the relation between receptor dynamics and the development of tolerance, experiments characterizing the entire time course of receptor and mRNA regulation as well as TAT dynamics are needed. Our follow-up studies were designed to answer these questions (36).

We monitored additional markers of adverse steroid effects including changes in body weight and different organ weights. In contrast to our previous acute dosing studies where there were reductions in body weights, we observed that the animals in our infusion study suffered substantial losses. Such effects could be the result of a combined effect of the steroid on several tissues. Tissues such as muscle, skin, lymphoid, and connective tissue are targets of the catabolic action of steroids, while liver is known to respond to steroids with anabolic effects reflecting an increased production of protein, RNA and glucose (37). An association between steroid therapy and liver abscesses was noted as early as in 1965 (38). Fatty liver is a well-known result of glucocorticoid therapy in rats (39) as well as humans (40). In agreement with these findings, we saw that there was a considerable hypertrophy of the liver in all treated rats. We noted that the lymphoid organs (spleen and thymus) showed a significant decrease in weight at the end of the 7-day infusion, which corroborates the well-known catabolic effects of steroids on these organs (37,41). One of the major side effects of chronic steroid therapy in humans is muscle wasting. All the treated rats in our study lost substantial muscle mass and similar effects upon administration of large doses of steroid have been reported earlier (42,43). The lungs were also subject to catabolic steroid effects. Glucocorticoids are known to produce glomerular lesions in man (44) and animals (45). However, no significant changes in kidney weights was noted in our study.

The rapid immunosuppressive effects in terms of blood lymphocyte trafficking upon single IV bolus dosing of prednisolone in male ADX Wistar rats was reported earlier (46). The IC_{50} we obtained was more than two-fold lower than the one reported in that paper (14.4 ng/mL). This supports the fact that MPL has greater immunosuppressive potency than prednisolone.

In summary, the present PK/PD model along with the results of the 7-day infusion study suggests that receptor regulation and dynamics are not altered upon long-term steroid treatment. The model can be used to derive steady-state receptor levels and the interaction of the activated nuclear receptor with the DNA can be employed to predict the extent of steroid mediated gene-induction upon acute and long-term dosing. Our results suggest that the extent of steady-state induction of various metabolic and immunosuppressive genes depends on the receptor occupancy, which in turn can be related to the steroid concentrations and dosage regimen. However, the final biological response or enzyme activity upon long-term continuous dosing is controlled not only by the direct enhancement of mRNA levels by the activated receptor but also on an upregulation in the signal transduction process as a result of other distinct biochemical and physiological changes caused by steroids. The extent and duration of steroid exposure thus play an important part in mediating steroid effects. Our current and previous studies indicate that the adrenalectomized rat seems to be an appropriate model to study the toxicity and beneficial effects of corticosteroids upon acute and long-term steroid dosing.

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Fig. 1.
Schematic representation of the cellular/molecular mechanism of steroid action in the hepatocyte. The thick open and solid arrows indicate induction and repression of gene transcription.

Fig. 2.

Fifth-generation model of acute corticosteroid receptor/gene-mediated effects. Symbols and differential equations for the model are defined in the text (Eqs. (7)–(16)). The dotted lines leading to the solid and open rectangles represent inhibition and induction of gene transcription by the drug-receptor complex in the nucleus DR(N) by indirect mechanisms.

Fig. 3.

Time course of receptor mRNA (top left panel), free cytosolic receptor density (bottom left panel), TAT mRNA (top right panel) and TAT activity (bottom right panel) upon single 50 (closed circles) and 10 mg/kg (open circles) IV bolus doses of methylprednisolone in male ADX Wistar rats. Symbols are mean data (Sun *et al.*, 1998) and vertical bars are the standard deviations. Solid and broken lines are fittings and simulations with the present model (Eqs. (7)–(16)) for the 50 and 10 mg/kg doses.

Fig. 4.

Time course of receptor mRNA (top left panel), free cytosolic receptor density (bottom left panel), TAT mRNA (top right panel) and TAT activity (bottom right panel) upon two 50 mg/kg IV bolus doses of methylprednisolone given at 0 and 24 hr in male ADX Wistar rats. Solid circles are mean data (Sun *et al.*, 1998), vertical bars are the standard deviations and solid lines are simulations with the fifth-generation model using Eqs. (7)–(16) and parameters listed in Table I.

Fig. 5.

Seven-day hepatic cytosolic receptor mRNA (top left panel), free receptor density (bottom left panel), TAT mRNA (top right panel) and TAT activity (bottom right panel) for the control and treatment groups administered the four infusion rates for seven days (vertical bar graphs). The * indicates significantly different from the control ($p < 0.05$). The horizontal lines indicate the means for each group. The horizontal broken lines are the predicted values using Eqs. (17)–(22) and parameters listed in Table I.

Fig. 6.

Simulations for the expected steady-state concentration–response relationship for methylprednisolone receptor/gene-mediated effects using Eqs. (17)–(22) and parameters listed in Table I (solid line). The effects of a 10-fold increase in k_{on} (long dashes), 10-fold decrease in k_{re} (dotted line), two-fold increase in S (short dashes), two-fold increase in γ (dash-dot-dash) on the responses are presented.

Fig. 7.

Blood lymphocyte count for each rat normalized by its own predose count at the end of the 7-day infusion period (vertical bars). The horizontal dashed lines are the group means as listed in Table II (R_{ss}) and used for calculation of IC_{50} with Eq. (25) derived from the cell trafficking model for redistribution of lymphocytes between the blood and lymphoid organs. The k_{in} and k_{out} are the zero- and first-order rate constants for transfer to lymphocytes to and from the blood pool. The solid rectangle indicates inhibition of lymphocyte egress from tissues based on the inhibition function in Eq. (23).

Table I

PK/PD parameters for receptor/gene-mediated steroid effects

Pharmacokinetics Parameter	Estimate	Parameter	Estimate
CL (L/hr/kg)	4.04 ^a /3.48 ^c	C_1 (ng/mL)	39,130 ^d
V_d (L/kg)	0.82 ^{a,b} /0.73 ^c	C_2 (ng/mL)	12,670 ^d
k_{12} (hr ⁻¹)	0.32 ^{a,b} /0.98 ^c	λ_1 (hr ⁻¹)	7.54 ^d
k_{21} (hr ⁻¹)	0.68 ^{a,b} /1.78 ^c	λ_2 (hr ⁻¹)	1.20 ^d

Receptor dynamics (fixed)	Value
k_{syn_Rm} (fmole/g/hr)	2.90
IC_{50_Rm} (nmole/L/mg protein)	26.2
k_{on} (L/nmole/hr)	0.00329
k_r (hr ⁻¹)	0.63
k_{re} (hr ⁻¹)	0.57
R_f	0.49
k_{dgr_R} (hr ⁻¹)	0.0572
R_{m0} (fmole/g)	25.8 ^d /18.6 ^{a,c} /4.74 ^b
R_0 (nmole/L/mg protein)	540.7 ^d /420 ^a /267 ^b /480.4 ^c

TAT Dynamics (estimated)	Value	CV%
S (L/nmole/mg protein)	0.0287	26.45
k_{dgr_tm} (hr ⁻¹)	0.383	27.35
γ	1.804	12.40
k_{dgr_t} (hr ⁻¹)	0.6904	43.97
TAT_{m0} (pmole/g)	0.2078 ^d /0.090 ^{a,c} /0.129 ^b	15.98
TAT_0 (A/mg protein)	0.0609 ^d /0.070 ^{a,b,c}	10.57

^a 10 mg/kg IV bolus.^b Infusion.^c 50 mg/kg IV bolus double dose.^d 50 mg/kg IV bolus single dose.

Table II

Lymphocyte trafficking parameters for methyl-prednisolone upon long-term dosing in male adrenalectomized rats.

Infusion Rate (mg/kg/hr)	C _{ss} (ng/mL)	R _{ss}	IC ₅₀ (ng/mL)
0.05	10 ^a	36.88	5.84
0.1	17.04	15.31	3.08
0.2	39.97	22.07	11.32
0.3	49.61	7.78	4.19

^a Assay limit of quantitation.