Published in final edited form as:

J Pharmacokinet Pharmacodyn. 2007 June ; 34(3): 333–354. doi:10.1007/s10928-007-9049-1.

Assessing the Dynamics of Nuclear Glucocorticoid-Receptor Complex: Adding Flexibility to Gene Expression Modeling¹

Anasuya Hazra², Debra C. DuBois^{2,3}, Richard R. Almon^{2,3}, and William J. Jusko^{2,4}

²Department of Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, State University of New York, 565 Hochstetter Hall, Buffalo, NY 14260, USA

³Department of Biological Sciences, University at Buffalo, State University of New York, Buffalo, NY 14260, USA

Abstract

A retrospective analysis was performed to modify our fourth-generation pharmacodynamic model for glucocorticoid receptor (GR) dynamics with incorporation of more physiological features. This modified model was developed by integrating previously reported free cytosolic GR and GR mRNA data following single (10, 50 mg/kg) and dual (50 mg/kg at 0 and 24 hr) intravenous doses of methylprednisolone (MPL) in adrenalectomized (ADX) male Wistar rats with several in vitro studies describing real-time kinetics of the transfer of rat steroid-receptor complex from the cell cytosol to the nucleus. Additionally, free hepatic cytosolic GR and its mRNA data from a chronic infusion dosing study of MPL (0.1 and 0.3 mg/kg/hr) in male ADX Wistar rats were used to verify the predictability of the model. Incorporation of information regarding in vitro receptor kinetics allowed us to describe the receptor-mediated pharmacogenomic effects of MPL for a larger variety of genes in rat liver from microarray studies. These included early responsive gene like CCAAT/enhancer binding protein- β (CEBP- β), a transcription factor, as well as the later responsive gene for tyrosine aminotransferase (TAT), a classical biomarker of glucocorticoid (GC) genomic effects. This more mechanistic model of GR dynamics can be applied to characterize profiles for a greater number of genes in liver.

Keywords

glucocorticoids; glucocorticoid receptor; nuclear localization; pharmacodynamics; methylprednisolone; pharmacogenomics

INTRODUCTION

Corticosteroids (CS) are widely used for the treatment of various inflammatory diseases, organ transplant patients, and as immunomodulatory agents (1, 2). Severe metabolic side effects, including steroid-induced diabetes, muscle atrophy, and disorders in lipid metabolism (3, 4), limit their therapeutic usefulness, especially with high-dose and long-

¹Supported by Grant GM 24211 from National Institute of Health

© 2007 Springer Science+Business Media, LLC

⁴To whom correspondence should be addressed: wjjusko@buffalo.edu.

term therapy. These actions of CS are predominantly generated by influencing the expression of diverse genes in numerous tissues. The regulation of gene expression by CS is mediated through the glucocorticoid receptor (GR) in target tissues.

Corticosteroids are protein-bound in plasma, predominantly associating with corticosteroid-binding globulin (CBG) and albumin (5). After dissociation from its plasma protein carrier, CS enter the cell by passive diffusion (6, 7) and associate with heat-shock protein bound cytosolic GR, causing its dissociation from the heat-shock protein complex. Subsequently, the drug-receptor complex becomes activated, hyperphosphorylated and undergoes conformational changes (1, 8). This activated drug-receptor complex translocates into the nucleus and binds to glucocorticoid response elements (GRE), specific DNA control regions (located at the 5' site) of the target gene, resulting in increased or decreased transcription of various mRNAs. This in turn results in translation to corresponding proteins and the specific physiological functions mediated by these proteins (9). The drug-receptor complex dissociates from the GRE and returns to the cytosol where it may be either degraded or dissociated to recycle the free receptors which in turn may bind to a new ligand (10). The glucocorticoids are also known to down-regulate their own receptors by a process called homologous down-regulation (11). Activated drug-receptor complex in the nucleus is known to inhibit the transcription of GR mRNA (12), thus further reducing the free cytosolic GR.

The effects of corticosteroids on the dynamics of free hepatic cytosolic receptor, receptor mRNA, and expression on target genes have been extensively studied in our lab. Mechanistic models were developed to capture the temporal patterns of receptor-mediated CS effects on various pharmacodynamic and pharmacogenomic (altered gene expression) markers (12–14). In these earlier studies, we were technically limited to studying relatively few target genes at a time, such as the up-regulation of hepatic TAT mRNA and activity, hepatic phosphoenol-pyruvate carboxykinase (PEPCK) mRNA and activity (15), glutamine synthetase (GS) mRNA and activity in the muscle (16), and down-regulation of GR mRNA. However, in microarray experiments (17, 18), where the effects of CS treatment were simultaneously examined for several thousands of genes, we encountered classes of rapidly up- or down-regulated genes which could not be described by our previous model. The nuclear drug-receptor complex (DR(N) in Fig. 1) which is assumed to mediate various pharmacogenomic effects of CS was not measured and was a calculated function. Previous models based on delayed gene effects of CS (TAT or GR mRNA) predicted that the concentration of DR(N) peaks about 2 hr following drug administration. Although simulated values of DR(N) could successfully be used as a driving force for up-regulation of later responsive genes, it could not explain CS modulation of genes which are up-regulated extremely rapidly. For example, CEBP- β , a transcription factor gene which has a recognized GRE in its promoter region and is known to be modulated by CS showed an increase in transcription within minutes of drug administration with a peak at 1 hr. Although transrepression by CS is not as well-characterized as transactivation, down-regulated genes in our microarray dataset such as aldehyde dehydrogenase (ADH), showed similar rapid down-regulation by CS, which could not be described by the DR(N) function.

The translocation of the steroid-receptor complex into the nucleus appears to be much faster than predicted by our earlier model. Various *in vitro* studies (10, 19) showed that 90% of

steroid-bound receptors can enter the cell nucleus from the cytosol within 5 min of incubation with 10 nM of dexamethasone (DEX). Hence, the time, predicted for the complete translocation of the receptor complex from our earlier model, was a considerable overestimation. Keeping the basic structural model, we have modified several aspects of our previous receptor dynamic model by incorporating literature information available and applied this modified model to CS-induced changes in expression of a large number of genes obtained from microarray studies.

MATERIALS AND METHODS

Experimental

Acute Dosing Studies—Data for determining hepatic GR and GR mRNA dynamics were obtained from our previous studies (12, 20). In brief, four groups of male adrenalectomized (ADX) Wistar rats were treated with MPL (Solu-Medrol®, The Upjohn Company, Kalamazoo, MI) via jugular vein cannula. One group of rats received a single dose of 50 mg/kg of MPL at 0 hr (12). In the second study three groups of rats received MPL at a single dose of 10 mg/kg at 0 hr, a single dose of 50 mg/kg at 0 hr, and dual doses of 50 mg/kg at 0 and 24 hr (20). Animals (3–4 per time point) were sacrificed over a period of 72 hr for the single dose groups (0.25, 0.5, 0.75, 1, 2, 4, 5, 5.5, 6, 7, 8, 12, 18, 24, 30, 36, 48 and 72 hr) and over a period of 120 hr (0.25, 1, 3, 5, 6, 7, 8, 12, 18, 24, 24.25, 25, 27, 29, 30, 31, 32, 36, 42, 54, 72, 96 and 120 hr) for the dual dose group. Separate groups of control rats were used for these two studies; these rats received saline through the cannula and were sacrificed throughout the course of the study and used as the 0 hr time point for the biomarkers.

Chronic Dosing Study—Data for the receptor and its mRNA during chronic dosing were obtained from another study (21). Two groups of male ADX Wistar rats were given 0.1 and 0.3 mg/kg/hr infusions of MPL using Alzet osmotic pumps (Model 2001, flow rate 1 μ l/hr; Alza, Palo Alto, CA) subcutaneously implanted between the shoulder blades. Rats were sacrificed at various times up to 7 days (6, 10, 13, 18, 24, 36, 48, 72, 96 and 168 hr). A control group of animals ($n = 4$) were implanted with saline-filled pumps and were sacrificed at various times throughout the 7-day study period.

For both the acute and chronic dosing studies, blood was collected, centrifuged, and the plasma was stored at -20°C for the analysis of MPL. Livers were harvested, flash frozen with liquid nitrogen, and stored at -80°C . Livers were ground to powder with a chilled mortar and pestle for further studies.

Microarray Study

The details of our microarray studies are published (17, 18). Briefly, total RNA from rat liver was extracted using Trizol reagent according to manufacturer's protocols. RNAs were quantified spectrophotometrically and integrity was assessed by gel electrophoresis. RNA samples were stored at -80°C . RNAs from individual rat livers were used to prepare the target and biotinylated cRNAs were hybridized to 47 individual Affymetrix GeneChips® Rat Genome U34A (Affymetrix, Inc.), containing 8799 probe sets. The values for each probe set

on each chip were normalized to the mean of the four control values for that probe, which allowed us to assess the fold change of genes of interest after treatment.

Assays

Plasma concentrations of MPL were determined by a normal phase high-performance liquid chromatography (HPLC) method (22). The lower limit of quantification is 10 ng/ml with inter- and intra-day assay variability less than 10%. A well-established radio-ligand binding assay was used to quantify the hepatic free cytosolic GR density. The total (D_T) and non-specific (D_{NS}) binding of radio-labeled DEX were measured (12, 23). The receptor density or B_{max} at any given time point was calculated by simultaneously solving Eqs. (1) and (2) using the ADAPT II program (24)

$$D_T = D_{NS} + \frac{D_f \cdot B_{max}}{D_f + K_D} \quad (1)$$

$$D_{NS} = K_{NS} \cdot D_f \quad (2)$$

where K_D is the equilibrium dissociation constant for receptor binding, K_{NS} is the non-specific binding constant and D_f is the free drug available to bind to receptors. The variability (CV%) of this assay was less than 15%. The receptor density values were normalized by protein content of the cytosol preparation (25). The GR mRNA was quantified by Northern hybridization (12, 26). For each animal, triplicate determinations were done and the CV% was less than 12%.

Modeling of Receptor Dynamics

Pharmacokinetics—The PK of MPL from all studies were described by bi-exponential kinetics. For this analysis the PK of MPL was fixed using parameters (Table I) from previous studies (12, 20)

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

where C_1 and C_2 represent intercepts and λ_1 and λ_2 represent slopes.

Receptor Dynamics—The model is based on the molecular mechanism by which CS are known to generate their genomic effects (Fig. 1). This model was applied to free cytosolic GR and its mRNA profiles.

MPL is known to bind to only albumin in the plasma yielding a stable protein-bound fraction of 0.77 for a wide range of total plasma concentrations (10–80,000 ng/ml) (27, 28). Therefore, it was assumed that only 23% of the plasma total drug enters the cell to bind to their cytosolic receptors. Thus the free drug can be expressed as

$$D_f = 0.23 \cdot D_t \quad (4)$$

where D_t is the total MPL concentration in the plasma in nM ($C_t \cdot 1000/374.4$; MW of MPL: 374.4), where C_t is expressed in terms of ng/ml and D_f is the intracellular drug concentration (in nM).

Since only the free drug can enter the cell by diffusion, it was assumed that the free drug (D_f) in the plasma binds to the hepatic free cytosolic receptor (R) forming the drug-receptor complex in the cytosol (DR). The DR then translocates into the nucleus of the cell forming DR(N) which is responsible for the down-regulation of GR mRNA by decreasing its synthesis. The dynamics of hepatic GR mRNA can be described by

$$\frac{dmRNA_{GR}}{dt} = k_{syn,GRmRNA} \cdot \left(1 - \frac{DR(N)}{IC_{50_Rm} + DR(N)}\right) - k_{dgr,GRmRNA} \cdot mRNA_{GR} \quad (5)$$

where IC_{50_Rm} is the level of DR(N) responsible for 50% inhibition of the transcription rate ($k_{syn,GRmRNA}$) of GR mRNA; and $k_{dgr,GRmRNA}$ is the first-order degradation rate constant for the mRNA. Assuming that in the absence of drug the receptor mRNA baseline $mRNA_{GR(0)}$ is constant, $k_{syn,GRmRNA}$ can be expressed as

$$k_{syn,GRmRNA} = k_{dgr,GRmRNA} \cdot mRNA_{GR(0)} \quad (6)$$

The free receptor in the cytosol, R , bound receptor, DR and DR(N), can be described as

$$\frac{dR}{dt} = k_{syn,GR} \cdot mRNA_R + R_f \cdot k_{re} \cdot DR(N) - k_{on} \cdot D_f \cdot R - k_{dgr,GR} \cdot R \quad (7)$$

$$\frac{dDR}{dt} = k_{on} \cdot D_f \cdot R - k_T \cdot DR \quad (8)$$

$$\frac{dDR(N)}{dt} = k_T \cdot DR - k_{re} \cdot DR(N) \quad (9)$$

where DR(N) is the drug bound receptor in the nucleus, $k_{dgr,GR}$ is the first-order degradation rate constant for GR, k_{on} is the association rate constant for steroid receptor binding, $k_{syn,GR}$ is a translational constant which is proportional to GR mRNA. Similar to receptor mRNA, it can be assumed that the baseline GR is constant and therefore the translational rate constant $k_{syn,GR}$ can be expressed as

$$k_{syn,GR} = \left(\frac{R_0}{mRNA_{GR(0)}}\right) \cdot k_{dgr,GR} \quad (10)$$

The values of the $mRNA_{GR(0)}$ and R_0 were obtained from Sun et al. (12, 20) and fixed for this analysis. The baseline values for DR and DR(N) are zero.

In Eq. (9), k_{re} is the overall turnover rate constant of DR(N) including the fraction (R_f) which is being recycled to form free R and the fraction $(1 - R_f)$, degraded; k_T is the first-order rate constant describing the translocation of DR to the nucleus. It has been reported that at the concentration of DEX equivalent to the intracellular concentration of MPL achieved even with the low-dose (10 mg/kg), the entry of DR in the nucleus is quite rapid after binding of steroid to its receptor; more than 90% of the bound receptor could potentially enter the nucleus within 5 min, which includes both the binding and the translocation process (10, 19). Hence, the value of k_T was fixed to 58.2 hr^{-1} ; considering nuclear translocation is complete within 5 min ($7 t_{1/2}$) to account for the rapid entry of DR to the nucleus.

The data from the high-dose, low-dose, and dual dose studies were fitted simultaneously to Eqs. (5)–(10) using ADAPT II (24) using the Maximum Likelihood method, where the variance model was

$$V(\sigma, \theta, t_i) = \sigma_1^2 \cdot Y(\theta, t_i)^{\sigma_2} \quad (11)$$

where Y represents the predicted value; σ_1 and σ_2 are the variance parameters which were fitted, and θ represents the structural parameters. The goodness of the fit was assessed by model convergence, visual inspection, Akaike Information Criterion (AIC), Schwarz Criterion (SC), estimator criterion value, and examination of residuals.

To check the predictability of our model, the parameters obtained from these fittings of receptor dynamics were used to simulate the mean and 95% confidence interval (CI) of the receptor time-profiles upon chronic dosing of MPL (at two infusion rates).

Array of Genomic Regulations by Nuclear Drug-Receptor Complex

Simulations were performed using indirect response (IDR) models (29) to describe a wide array of genomic responses that can be generated using DR(N) as the driving force

$$\frac{dmRNA_s}{dt} = k_{syn}(1 + S_{sim}DR(N)) - k_{deg} \cdot mRNA_s \quad \text{Model III} \quad (12)$$

$$\frac{dmRNA_I}{dt} = k_{syn} \left(1 - \frac{DR(N)}{DR(N) + IC_{50sim}} \right) - k_{deg} \cdot mRNA_I \quad \text{Model I}, \quad (13)$$

where $mRNA_s$ and $mRNA_I$ genes which are stimulated and inhibited upon CS administration, k_{syn} is the zero-order production rate, k_{deg} is the first-order loss rate for the respective mRNA, S_{sim} is a linear stimulation constant, and IC_{50sim} is the concentration of DR(N) required to produce 50% inhibition of k_{syn} . The genes which are simply stimulated (Model III) or inhibited (Model I) upon CS administration may have different time profiles based on their turnover rate (k_{deg}) or drug related parameters, i.e., the stimulation constant S_{sim} (Eq. 12) or IC_{50sim} . A wide range of responses were simulated using various values of k_{deg} , S_{sim} (Model III) and IC_{50sim} for Model I.

Modeling of Up- and Down-Regulated mRNA

The mRNA time profiles for hepatic TAT, CEBP- β , ornithine decarboxylase (ODC), argininosuccinate synthetase (ASS), aldehyde dehydrogenase (ADH), low density lipoprotein receptor (LDL-R), carbonic anhydrase (CA) and glutaminase mRNA from our microarray study (17, 18) from the high-dose group (50 mg/kg single dose) of MPL treated animals are examples of rapid and slow responding genes. Equation (12) was used to describe the increase in transcription of TAT, CEBP- β , ODC and ASS mRNA. Equation (13) was used for down-regulation in transcription of both ADH, LDL-R, CA and GA mRNA. In both cases DR(N) was used as the driving force to regulate transcription these genes. The mRNAs of interest, mRNA_s, are produced with a zero-order transcription rate constant, k_{syn} and degraded with the first-order rate constant, k_{deg} . The base-line is

$$\text{mRNA}_s = \frac{k_{\text{syn}}}{k_{\text{deg}}} \quad (14)$$

Since the signal from each chip was normalized by dividing by the mean of the signals from the control chips, the baselines for all mRNAs in the microarray dataset starts from 1. For some genes the baselines were estimated for improvement of model fitting.

A piecewise approach was used for fitting the pharmacogenomic data. The PK of MPL was fixed in fitting the receptor dynamics from all dose levels. Subsequently all the PK and receptor parameters were fixed in fitting the mRNA profiles for all the up- and down-regulated genes used for fittings.

RESULTS

Pharmacokinetics

The simulated PK profiles for MPL are shown in Fig. 3a. The PK of MPL was described by bi-exponential kinetics for all dose levels. The half-lives of MPL for both the high-dose and the dual doses are approximately 30 min; however, for the 10 mg/kg dose the $t_{1/2}$ is 1.1 hr. Different parameter values were used for different dose levels (12, 20), which are given in Table I.

Pharmacodynamic Analysis

GR mRNA—The observed steroid receptor mRNA dynamics after various doses of MPL are shown in Fig. 2 (top panel) along with mean and 95% CI of the model (Fig. 1) predictions. The drug causes down-regulation of the receptor mRNA at all dose levels. Variability in mRNA is apparent especially in the single low (10 mg/kg) and dual dose groups; however, most of the observed data fell within the 95% CI of model predictions. The model parameters could be estimated with reasonable precision (7–56%) (Table II). The baselines for the GR mRNA were different in rats from the different dose groups and were fixed for this analysis since estimation of the baselines did not improve the overall model fitting. The synthesis ($k_{\text{syn,GRmRNA}} = 1.96\text{--}3.2 \text{ fmol g}^{-1} \text{ hr}^{-1}$), and the degradation ($k_{\text{dgr,GRmRNA}} = 0.122 \text{ hr}^{-1}$, 24% variability), rate constants, for the GR mRNA are very similar to values from Sun et al. of $1.77\text{--}2.9 \text{ fmol g}^{-1} \text{ hr}^{-1}$ and 0.11 hr^{-1} (35% variability)

(12, 20). The present value of IC_{50_Rm} i.e. DR(N) required to cause 50% reduction in the GR mRNA was somewhat higher than previously reported, e.g. 26.2 (50 mg/kg single dose, CV% not reported) and 50.7 (dual and low-dose study, variability 63%) nmol/l/mg of protein compared to 123.7 nmol/l/mg protein (55.7% variability). However, considering the variability of these estimated parameter, they were not markedly different.

Receptor Dynamics—Observed hepatic cytosolic free GR density time profiles along with the model predictions are shown in Fig. 2 (bottom panel) from all acute dose groups. Since the protein binding of MPL (77%) was considered and only the free drug was assumed to interact with the receptor, the estimated value of k_{on} ($1.9 \cdot 10^{-2} \text{ nM}^{-1} \text{ hr}^{-1}$, 47.8% CV) was somewhat higher ($3.29 \cdot 10^{-3} \text{ nM}^{-1} \text{ hr}^{-1}$, acute high-dose study, no CV% reported, $4.87 \cdot 10^{-4} \text{ nM}^{-1} \text{ hr}^{-1}$, low and dual dose studies, 25% CV) than previously reported values (12, 20).

The free receptors from the cytosol disappear very rapidly after administration of the drug irrespective of the dose. However, for higher doses these levels remain suppressed for a longer period of time (4–5 hr) compared to the low-dose group where the receptor level begins to increase almost immediately. In both cases, biphasic return of receptors is apparent, which could partly be accounted for by receptor recycling and partly to *de novo* synthesis of receptor from its mRNA, since the slower return phase for the receptor parallels the return of the GR mRNA. At the end of the first rapid return phase (approximately 10 hr), receptors return to almost 40–50% of the baseline, which is somewhat lower than the estimated recycling factor, $R_f = 0.69$ (95% CI: 0.59–0.79). This may imply that a portion of receptors undergo binding and recycling more than once. The second slower phase could be attributable to both receptor synthesis as well as recycling. The complete recovery of basal levels of receptors occurs at around 72 hr after both the 10 and 50 mg/kg doses.

DR and DR(N) Dynamics—Cell culture studies show that the disappearance of drug-bound receptor from the cytosol and its subsequent entry into the nucleus is very rapid (10, 19). Therefore, the transfer rate constant k_T was fixed to 58.2 hr^{-1} , which is equivalent to seven half-lives of the translocation rate. Whereas the cytosol to nuclear transfer of the steroid-receptor complex is quite rapid, the process of nuclear to cytosolic return is much slower (10). This process was nicely captured by the simulated profiles for DR(N) from our model (Fig. 3). Although the drug-bound receptor reached its maximum inside the nucleus within 5 min following MPL dosing, complete dissipation of this moiety from the nucleus required almost 10 hr. For the dual dose study, it can be observed that the free receptor levels in the cytosol do not return to the baseline before the second 50 mg/kg dose. Therefore, the subsequent formation of DR and DR(N) in the nucleus are lower after the second dose.

Chronic Dosing—Based on the parameters (with variability) obtained from the simultaneous fitting of the free cytosolic receptor and mRNA from all the single and dual-dosing studies, simulations were performed to predict the profiles (mean and 95% CI) after chronic dosing, which are shown in Fig. 4. Both GR mRNA and free cytosolic receptors remain down-regulated for the entire time during the treatment. Our model was able to predict this behavior quite well, especially for the free cytosolic receptor profile. Similar to

the acute dosing studies, much variability was evident in the GR mRNA profiles. The simulated profiles for the drug-receptor complex both in the cytosol and nucleus show an early rapid up-regulation; however, they eventually decline to a different steady-state than the baseline reflecting a new homeostasis.

Pharmacogenomic Modeling—Simulations shown in Fig. 5a,b show a wide range of responses that can be generated from our improved DR(N) profiles, with some genes being affected by the drug treatment much quicker than others based on their respective turnover rate constants. Particularly with the higher values of k_{deg} (while $\text{IC}_{50\text{sim}}$ or S_{sim} remains constant) the response increases much faster than the lower values and thus could explain the variations in onset of responses for various genes. Further flexibility in the responses arise from differences is the ability of the drug to produce an effect, described by various stimulation or inhibitory constants (S_{sim} or $\text{IC}_{50\text{sim}}$). For the inhibitory responses it was assumed case that drug was able to cause maximum inhibition of the system ($I_{\text{max}} = 1$).

The mRNAs for CEBP- β , TAT, ODC and ASS showed up-regulation following 50 mg/kg MPL as depicted in Fig. 6. Although these increases in transcription were evident within 15 min (except ASS) of drug exposure, the effect on CEBP- β mRNA (peaking at 1–2 hr) is much faster than TAT or ODC mRNA (peaking at 5–6 hr), and was latest for ASS mRNA (8–10 hr). This can be attributed to the difference in their loss rate constants, 0.047 hr^{-1} (40.6% CV) for ASS, 0.436 hr^{-1} (35.3% CV) for TAT mRNA compared to 1.66 hr^{-1} (28% CV) for CEBP- β . Stimulation constants ($S_{\text{DR(N)}}$) were comparable between CEBP- β and TAT, however, for ASS and ODC they were somewhat higher, perhaps due to the extent of stimulation that was induced by DR(N) compared to TAT and CEBP- β . It should be noted that extent of stimulation for ASS (~2-fold from the baseline) was as high as for TAT (~2-fold) or CEBP- β (~3.5-fold), although the loss rate constant was much lower.

Differences in k_{deg} were seen for the down-regulated genes (Fig. 7), i.e. 3.51 hr^{-1} (40.7% CV) for ADH, 1.88 hr^{-1} for LDL-R, 1.31 hr^{-1} for CA compared to a much lower value for GA, 0.139 hr^{-1} (11% CV), whereas the $\text{IC}_{50\text{sim}}$ values were closer. Thus we observed a more rapid down-regulation for ADH, LDL-R and CA compared to GA. The DR(N) function derived from our model was able to capture all of these profiles adequately with reasonable precision for all estimated parameters (Tables III, IV). Model convergence could not be achieved when attempts were made to use the previous model derived DR(N) to describe the rapidly up- or down-regulated genes. The present degradation rate constant for TAT mRNA (0.436 hr^{-1} , 35.3% CV) very closely resembled the value (0.383 hr^{-1} , 27.4% CV) estimated previously for the TAT mRNA profile obtained by Northern Hybridization (21).

Discussion

For the past two decades, our laboratory has been involved in developing mechanism-based models of various receptor-mediated effects of corticosteroids. This report was aimed at modifying some aspects of our previous models for CS receptor dynamics. Our fourth-generation model (12, 20) for receptor dynamics was built based on treating ADX rats with various doses of MPL and examining effects on hepatic free cytosolic receptors and hepatic

GR mRNA. It has been reported (1, 2, 8, 30) that most CS genomic effects are mediated by their drug-bound receptor dimer in the cell nucleus. Both the cytosolic (DR) as well as nuclear (DR(N)) drug-bound receptors, although they could not be quantitatively measured in any of our studies, are essential components of our mechanistic models. Thus in previous reports parameters associated with these two model compartments were based on the data available for the drug, its receptor, and the GR mRNA. The transduction compartment, DR(N), was used to model many CS driven effects, such as changes in hepatic TAT mRNA and activity (12, 14, 20, 21), hepatic PEPCK mRNA and activity (15), and many other genomic effects of CS (18). Since these effects are generally of slow onset (i.e. peaks at 2 hr and later), the DR(N) peak, 2 hr post dose estimated from the fourth-generation model was able to capture the dynamics of most of these effects. However, this DR(N) profile was unable to explain drug effects on genes which are affected by CS much faster than 2 hr (e.g. CEBP- β mRNA, a transcription factor, known to have GC response element in its promoter region and which becomes up-regulated within 15 min after MPL treatment peaking at around 1 hr) or ADH mRNA, an enzyme mRNA reported to be influenced by CS treatment (31, 32). This led us to investigate the kinetics of DR and DR(N) more carefully, i.e., how rapidly the receptor translocates from the cytosol to the nucleus of the cell once it is bound to its ligand. Many *in vitro* studies with rat glucocorticoid receptors monitoring the transfer of steroid receptors from the cell cytosol to the nucleus (10, 33, 34) showed that DEX binding resulted in the transfer of the drug-bound receptor to the nucleus is nearly complete (seven half-lives for 99% completion, $k_T \sim 58.2 \text{ hr}^{-1}$) within 5–10 min of steroid incubation. This transfer is both ligand- and concentration-dependent. The free MPL concentrations in the hepatic cells in our studies, even after of the low-dose (10 mg/kg), approximated about 1000 nM. This high concentration of MPL may trigger the cytosol-nuclear transfer of steroid receptor even faster than observed in the *in vitro* studies. Thus fixing the value of k_T to 58.2 hr^{-1} allowed the DR(N) complex to form much faster than predicted by our previous model. Remaining parameters could be estimated with reasonable precision yielding good fittings of GR receptor density and mRNA data from all dose levels. However, this raised the question of the importance of k_T on the outcome of the model fittings since use of different sets of k_T values, i.e., 0.63 or 2.03 hr^{-1} from previous fittings (12, 20) or use of an *in vitro* value (58.2 hr^{-1}) yielded comparable fittings of GR density and GR mRNA profiles. A sensitivity analysis revealed that changing the value of k_T beyond 0.6 hr^{-1} does not change the predicted levels of GR density and GR mRNA significantly when other parameters are kept constant. On the other hand, varying k_T beyond 0.6 hr^{-1} affects the dynamics of DR or DR(N) but only to 15 hr^{-1} , beyond which it does not allow any faster transfer from cytosol (DR) to the nuclear (DR(N)) compartment. Since the mechanism of action of genomic effects of CS indicates the presence of both DR and DR(N) compartments in the simplest case, we retained the basic structure of the model. However, due to the apparent insensitivity of the GR mRNA and GR density to higher values, k_T was fixed to the *in vitro* values and the rest of the parameters were estimated.

The PK parameters obtained from the previous studies were fixed for this analysis unlike the receptor dynamics which were fitted simultaneously from all dose levels. Although the PK of MPL apparently can be captured by bi-exponential kinetics, there are differences in half-lives between low and high doses. The PK of MPL shows dose-dependent changes in

volume of distribution and apparent clearance along with reversible metabolism between methylprednisolone and its inactive metabolite methylprednisone in rats (35). Thus for simplicity we used basic bi-exponentials as forcing functions to describe the PK after various doses of MPL. Determination of MPL in plasma (22) measures total plasma drug concentration, whereas only the free drug can enter the cell and bind to the receptor. In the previous studies (12, 20), total MPL in the plasma was allowed to interact with the intracellular receptors, which perhaps resulted in an apparent binding constant, k_{on} , which is somewhat lower (since the amount of cytosolic receptors are fixed) than the true value. Protein binding of MPL in rat plasma is linear since it only binds to albumin. Hence, use of the free fraction of the drug (0.23) allowed correction for a possible underestimation (~10-fold lower) of the binding constant, k_{on} , from previous studies. However, the k_{on} estimated in our analysis is a combination of change in k_t , subsequent change in R_f , protein binding, and perhaps intracellular efflux and metabolism.

Although our comprehensive and mechanistic model describing steroid receptor dynamics was re-evaluated with protein binding of the drug and the rapid entry of the drug bound receptor into the nucleus, the overall model structure was not changed from the fourth- (12, 20) and fifth-generation models (14, 21). Our modified model is highly flexible in describing both early and late responsive genes. Difficulties were obviated in achieving model convergence when the former DR(N) attempted fitting early responsive genes. This problem also prevented making statistical comparisons between the previous and current models. For the delayed effects, however, no significant improvement was observed for the newer model compared to the former one in terms of the usual model fitting criteria. The parameters associated with the genomic components were based on only the high-dose of MPL and one should be cautious when attempting to extrapolate these predictions to other dose levels.

The present model of receptor dynamics, however, is more mechanistically based and able to account for previous pharmacogenomic profiles (GS, TAT, and PEPCK) as well as for an enormous array of other genes which have been quantified using microarray methods. Profiles of thousands of genes altered by MPL in various rat tissues are available online (<http://www.pepr.cnmcresearch.org>).

References

1. Schacke H, Docke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Therap.* 2002; 96:23–43. [PubMed: 12441176]
2. Wilcke JR, Davis LE. Review of glucocorticoid pharmacology. *Vet Clin North Am – Small Anim Pract.* 1982; 12:3–17. [PubMed: 7048712]
3. Belvisi MG, Wicks SL, Battram CH, Bottoms SE, Redford JE, Woodman P, Brown TJ, Webber SE, Foster ML. Therapeutic benefit of a dissociated glucocorticoid and the relevance of in vitro separation of transrepression from transactivation activity. *J Immunol.* 2001; 166:1975–1982. [PubMed: 11160246]
4. Maxwell SR, Moots RJ, Kendall MJ. Corticosteroids: Do they damage the cardiovascular system? *Postgrad Med J.* 1994; 70:863–870. [PubMed: 7870631]
5. Czock D, Keller F, Rasche FM, Haussler U. Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. *Clin Pharmacokinet.* 2005; 44:61–98. [PubMed: 15634032]

6. Dostert A, Heinzel T. Negative glucocorticoid receptor response elements and their role in glucocorticoid action. *Curr Pharm Des.* 2004; 10:2807–2816. [PubMed: 15379669]
7. Vamvakopoulos NO. Tissue-specific expression of heat shock proteins 70 and 90: Potential implication for differential sensitivity of tissues to glucocorticoids. *Mol Cell Endocrinol.* 1993; 98:49–54. [PubMed: 8143913]
8. Schoneveld OJ, Gaemers IC, Lamers WH. Mechanisms of glucocorticoid signalling. *Biochim Biophys Acta.* 2004; 1680:114–128. [PubMed: 15488991]
9. Swartz SL, Dluhy RG. Corticosteroids: Clinical pharmacology and therapeutic use. *Drugs.* 1978; 16:238–255. [PubMed: 209958]
10. Hache RJ, Tse R, Reich T, Savory JG, Lefebvre YA. Nucleocytoplasmic trafficking of steroid-free glucocorticoid receptor. *J Biol Chem.* 1999; 274:1432–1439. [PubMed: 9880517]
11. Oakley RH, Cidlowski JA. Homologous down regulation of the glucocorticoid receptor: The molecular machinery. *Crit Rev Eukaryot Gene Expr.* 1993; 3:63–88. [PubMed: 8324294]
12. Sun YN, DuBois DC, Almon RR, Jusko WJ. Fourth-generation model for corticosteroid pharmacodynamics: A model for methylprednisolone effects on receptor/gene-mediated glucocorticoid receptor down-regulation and tyrosine amino-transferase induction in rat liver. *J Pharmacokinet Biopharm.* 1998; 26:289–317.
13. Haughey DB, Jusko WJ. Receptor-mediated methylprednisolone pharmacodynamics in rats: Steroid-induced receptor down-regulation. *J Pharmacokinet Biopharm.* 1992; 20:333–355. [PubMed: 1362219]
14. Ramakrishnan R, DuBois DC, Almon RR, Pyszczynski NA, Jusko WJ. 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. *J Pharmacokinet Pharmacodyn.* 2002; 29:1–24. [PubMed: 12194533]
15. Jin JY, DuBois DC, Almon RR, Jusko WJ. Receptor/gene-mediated pharmacodynamic effects of methylprednisolone on phosphoenolpyruvate carboxykinase regulation in rat liver. *J Pharmacol Exp Ther.* 2004; 309:328–339. [PubMed: 14722324]
16. Sun YN, McKay LI, DuBois DC, Jusko WJ, Almon RR. Pharmacokinetic/pharmacodynamic models for corticosteroid receptor down-regulation and glutamine synthetase induction in rat skeletal muscle by a receptor/gene-mediated mechanism. *J Pharmacol Exp Ther.* 1999; 288:720–728. [PubMed: 9918581]
17. Almon RR, Dubois DC, Jin JY, Jusko WJ. Temporal profiling of the transcriptional basis for the development of corticosteroid-induced insulin resistance in rat muscle. *J Endocrinol.* 2005; 184:219–232. [PubMed: 15642798]
18. Jin JY, Almon RR, DuBois DC, Jusko WJ. Modeling of corticosteroid pharmacogenomics in rat liver using gene microarrays. *J Pharmacol Exp Ther.* 2003; 307:93–109. [PubMed: 12808002]
19. Htun H, Barsony J, Renyi I, Gould DL, Hager GL. Visualization of glucocorticoid receptor translocation and intranuclear organization in living cells with a green fluorescent protein chimera. *Proc Natl Acad Sci USA.* 1996; 93:4845–4850. [PubMed: 8643491]
20. Sun YN, DuBois DC, Almon RR, Pyszczynski NA, Jusko WJ. Dose-dependence and repeated-dose studies for receptor/gene-mediated pharmacodynamics of methylprednisolone on glucocorticoid receptor down-regulation and tyrosine amino-transferase induction in rat liver. *J Pharmacokinet Biopharm.* 1998; 26:619–648. [PubMed: 10485078]
21. Ramakrishnan R, DuBois DC, Almon RR, Pyszczynski NA, Jusko WJ. Pharmacodynamics and pharmacogenomics of methylprednisolone during 7-day infusions in rats. *J Pharmacol Exp Ther.* 2002; 300:245–256. [PubMed: 11752123]
22. Haughey DB, Jusko WJ. Analysis of methylprednisolone, methylprednisone and corticosterone for assessment of methylprednisolone disposition in the rat. *J Chromatogr.* 1988; 430:241–248. [PubMed: 3235500]
23. Boudinot FD, D'Ambrosio R, Jusko WJ. Receptor-mediated pharmacodynamics of prednisolone in the rat. *J Pharmacokinet Biopharm.* 1986; 14:469–493. [PubMed: 2879901]
24. D'Argenio, DS.; Schumitzky, A. ADAPT II User's Guide: Pharmacokinetic/pharmacodynamic Systems Analysis Software. Biomedical Simulations Resource; LA: 1997.

25. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951; 193:265–275. [PubMed: 14907713]
26. DuBois DC, Almon RR, Jusko WJ. Molar quantification of specific messenger ribonucleic acid expression in northern hybridization using cRNA standards. *Anal Biochem.* 1993; 210:140–144. [PubMed: 7683846]
27. Ebling WF, Milsap RL, Szefer SJ, Jusko WJ. 6 alpha-Methylprednisolone and 6 alpha-methylprednisone plasma protein binding in humans and rabbits. *J Pharm Sci.* 1986; 75:760–763. [PubMed: 3772747]
28. Haughey DB, Jusko WJ. Receptor-mediated methylprednisolone pharmacodynamics in rats: Steroid-induced receptor down-regulation. *J Pharmacokinet Biopharm.* 1992; 20:333–355. [PubMed: 1362219]
29. Dayneka NL, Garg V, Jusko WJ. Comparison of four basic models of indirect pharmacodynamic responses. *J Pharmacokinet Biopharm.* 1993; 21:457–478. [PubMed: 8133465]
30. Newton R. Molecular mechanisms of glucocorticoid action: What is important? *Thorax.* 2000; 55:603–613. [PubMed: 10856322]
31. Falkner KC, Xiao GH, Pinaire JA, Pendleton ML, Lindahl R, Prough RA. The negative regulation of the rat aldehyde dehydrogenase 3 gene by glucocorticoids: Involvement of a single imperfect palindromic glucocorticoid responsive element. *Mol Pharmacol.* 1999; 55:649–657. [PubMed: 10101022]
32. Lindahl R, Xiao GH, Falkner KC, Prough RA. Negative regulation of rat hepatic aldehyde dehydrogenase 3 by glucocorticoids. *Adv Exp Med Biol.* 1999; 463:159–164. [PubMed: 10352681]
33. Hager GL, Smith CL, Fragoso G, Wolford R, Walker D, Barsony J, Htun H. Intracellular trafficking and gene targeting by members of the steroid/nuclear receptor superfamily. *J Steroid Biochem Mol Biol.* 1998; 65:125–132. [PubMed: 9699865]
34. Sackey FN, Hache RJ, Reich T, Kwast-Welfeld J, Lefebvre YA. Determinants of subcellular distribution of the glucocorticoid receptor. *Mol Endocrinol.* 1996; 10:1191–1205. [PubMed: 9121487]
35. Haughey DB, Jusko WJ. Bioavailability and nonlinear disposition of methyl-prednisolone and methylprednisone in the rat. *J Pharm Sci.* 1992; 81:117–121. [PubMed: 1545348]

Fig. 1.
Model for CS pharmacokinetics and receptor dynamics. The symbols are defined in Eqs. (3)–(9) and Table II.

Fig. 2.

Hepatic GR mRNA (upper panel) and free cytosolic GR (lower panel) after MPL administration at doses of (a) 10 mg/kg at 0 hr, (b) 50 mg/kg at 0 hr and (c) 50 mg/kg at 0 and 24 hr. The symbols represent the observed data and the solid and the broken lines represent the mean and 95% CI of model predictions.

Fig. 3.

(a) Simulated pharmacokinetic profile of MPL, (b) simulated hepatic drug-receptor complex in the cytosol and (c) in the nucleus, after the indicated doses of MPL.

Fig. 4.

Hepatic concentrations of (a) GR mRNA, (b) free cytosolic GR and (c) simulated drug-bound receptor complex in the cytosol (broken line) and nucleus (solid line) versus time after the indicated doses of MPL.

Fig. 5.

Simulation of various stimulatory (top) and inhibitory (bottom) responses. The solid lines depict the cases where k_{deg} (0.5) was constant and values of S_{sim} (0.002, 0.01, 0.03, 0.05) or IC_{50sim} (10, 50, 200, 500) were varied; the broken lines indicate where S_{sim} (0.02) and IC_{50sim} (100) were constant and k_{deg} (0.01, 0.05, 0.1, 1.0 for Model III and 0.1, 0.3, 0.8 and 2.0 for Model I) were varied.

Fig. 6.

Dynamics of hepatic mRNA (up-regulated) for CEBP- β , TAT, ODC and ASS from a microarray dataset after 50 mg/kg of MPL. The symbols depict the mean $\pm$ SD of observed data and solid lines represent model fittings.

Fig. 7.

Dynamics of hepatic mRNA (down-regulated) for ADH, LDL-R, CA and GA from a microarray dataset after 50 mg/kg of MPL. The symbols depict the mean $\pm$ SD of observed data and solid lines represent model fittings.

Table I**Pharmacokinetic Parameters of Methylprednisolone**

Parameters	Definition	Values
C_1 (ng/ml)	Intercept	39, 130 ^a , 12, 060 ^b , 11, 300 ^c
C_2 (ng/ml)	Intercept	12, 670 ^a /130 ^b /5740 ^c
λ_1 (hr ⁻¹)	Slope	7.54 ^a /5.30 ^b /6.15 ^c
λ_2 (hr ⁻¹)	Slope	1.2 ^a /0.63 ^b /1.38 ^c
$t_{1/2}$ (hr)	Half-life	0.58 ^a /1.1 ^b /0.5 ^c
V_c (l/Kg)	Central volume	0.97 ^a /0.82 ^b /0.73 ^c
V_{ss} (l/Kg)	Steady-state volume	1.9 ^a /1.21 ^b /1.13 ^c

^aFor 10 mg/kg MPL.

^bFor 50 mg/kg MPL dual dose.

^cFor 50 mg/kg MPL single dose.

Table II

Receptor Dynamic Parameters from Simultaneous Fittings of Hepatic Free Cytosolic Receptor Densities and Receptor mRNA

Receptor Dynamics	Definition	Value (CV%)
$k_{s,GRmRNA}$ (fmol/g/hr)	GR mRNA synthesis rate constant	2.1 (11.5) ^a /1.96 (39.4) ^b /3.2 (35.1) ^c
$k_{dgr,GRmRNA}$ (hr ⁻¹)	GR mRNA degradation rate constant	0.122 (24.0)
IC _{50_Rm} (nmol/l/mg protein)	DR(N) required for 50% inhibition of GR mRNA synthesis	123.7 (55.7)
k_{on} (l/nmol/hr)	Association constant for steroid GR binding	0.019 (47.8)
k_T (hr ⁻¹)	Translocation constant	58.2 (Fixed)
k_{re} (hr ⁻¹)	Degradation constant for DR(N)	0.402 (35.1)
R_f	Recycling factor constant	0.69 (7.1)
$k_{syn,GR}$ (nmol/l/mg protein/fmole GRmRNA/g/hr)	GR synthesis rate constant	0.88 (9.2) ^a /1.2 (10.1) ^b /0.84 (11.4) ^c
$k_{dgr,GR}$ (hr ⁻¹)	GR degradation rate constant	0.0403 (28.2)
$mRNA_R^0$ (fmol/g) _(fmol/g)	Hepatic GR mRNA baseline	17.24 (11.5) ^a /16.05 (39.4) ^b /25.8 (35.1) ^c
R^0 (fmol/mg protein)	Hepatic free cytosolic GR baseline	375.1 (9.2) ^a /480.4 (10.1) ^b /540.7 (11.4) ^c

^aFor 10 mg/kg MPL.

^bFor 50 mg/kg MPL dual dose.

^cFor 50 mg/kg MPL single dose.

Table III

Dynamic Parameters for Four Hepatic Up-regulated Genes

Parameters	Definitions	TAT	CEBP- β	ODC	ASS
$k_{deg,BM}$ (hr^{-1})	First-order loss rate constant	0.436 (35.3)	1.66 (28.0)	0.214 (22.6)	0.047 (40.6)
S_{sin} ($fmol/mg\ protein^{-1}$)	Linear stimulation constant by DR(N)	0.003 (26.7)	0.004 (15.5)	0.024 (11.4)	0.013 (48.6)
mRNA ₀	Baseline	0.9 (10.3)	1.06 (5.4)	1.0 (fixed)	0.83 (13.8)

TAT, tyrosine aminotransferase; CEBP- β , CCAAT-enhancer binding protein beta; ODC, ornithine decarboxylase; ASS, argininosuccinate synthetase.

Table IV

Dynamic Parameters for Four Hepatic Down-regulated Genes

Parameters	Definitions	CA	ADH	LDL-R	GA
$k_{deg,BM}$ (hr^{-1})	First-order loss rate constant	1.31 (23.5)	3.51 (40.7)	1.88 (34.3)	0.139 (11.0)
$IC_{50\ sim}$ (nM)	Concentration of 50% inhibition	403.5 (12.1)	200.8 (25.0)	205.3 (23.6)	59.9 (30.2)
mRNA ₀	Baseline	1.0 (fixed)	1.0 (6.2)	1.0 (fixed)	1.0 (fixed)

ADH, aldehyde dehydrogenase; LDL-R, low density lipoprotein receptor; GA, glutaminase; CA, carbonic anhydrase.