Modeling Corticosteroid Effects in a Rat Model of Rheumatoid Arthritis II: Mechanistic Pharmacodynamic Model for Dexamethasone Effects in Lewis Rats with Collagen-Induced Arthritis

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ABSTRACT

A mechanism-based model for pharmacodynamic effects of dexamethasone (DEX) was incorporated into our model for arthritis disease progression in the rat to aid in identification of the primary factors responsible for edema and bone loss. Collagen-induced arthritis was produced in male Lewis rats after injection of type II porcine collagen. DEX was given subcutaneously in single doses of 0.225 or 2.25 mg/kg or 7-day multiple doses of 0.045 or 0.225 mg/kg at 21 days postdisease induction. Effects on disease progression were measured by paw swelling, bone mineral density (BMD), body weights, plasma corticosterone (CST), and tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and glucocorticoid receptor (GR) mRNA expression in paw tissue. Lumbar and femur BMD was determined by PIXIImus II dual-energy X-ray absorptiometry. Plasma CST was assayed by high-performance liquid chromatography. Cytokine and GR mRNA were assayed by quantitative real-time polymerase chain reaction. Indirect response models, drug interaction models, transduction processes, and the fifth-generation model of corticosteroid dynamics were integrated and applied using S-ADAPT software to describe how dexamethasone binding to GR can regulate diverse processes. Cytokine mRNA, GR mRNA, plasma CST, and paw edema were suppressed after DEX administration. TNF-α mRNA expression and BMD seemed to increase immediately after dosing but were ultimately reduced. Model parameters indicated that IL-6 and IL-1β were most sensitive to inhibition by DEX. TNF-α seemed to primarily influence edema, whereas IL-6 contributed the most to bone loss. Lower doses of corticosteroids may be sufficient to suppress the cytokines most relevant to bone erosion.

Corticosteroids (CS) have long played a role in treatment of rheumatoid arthritis (Hart, 1976; Neeck, 2002). Therapy with this class of drugs dates back to early 1950 before quantitative methods describing the kinetics and dynamics of drug disposition and treatment effects were considered necessary for drug development and clinical applications. Numerous adverse effects became recognized and thus dose adjustments were often warranted and other drugs were sought to replace chronic CS treatment regimens. However, CS have remained a mainstay in treatment when other drugs fail and often for bridging between alternative treatments.

Furthermore, recent studies using low-dose prednisolone or methylprednisolone (MPL) alone or in combination with other small-molecule drugs have highlighted the beneficial role of these compounds in preventing irreversible joint erosion (Svensson et al., 2005; Wassenberg et al., 2005; Da Silva et al., 2006). In the present study, quantitative analysis of CS effects on proinflammatory cytokine mRNA in a rat model of rheumatoid arthritis was performed and used to explain differences in concentrations necessary for treatment effects on paw edema versus bone mineral density (BMD), potentially providing insight for optimizing the use of CS in the clinical setting.

A disease progression model for proinflammatory cytokine mRNA, glucocorticoid receptor (GR) mRNA, plasma corticosterone (CST), paw edema, and BMD is presented in Earp et al. (2008). Here, we integrate the effects of dexamethasone

ABBREVIATIONS: CS, corticosteroid(s); MPL, methylprednisolone; BMD, bone mineral density; GR, glucocorticoid receptor; CST, corticosterone; DEX, dexamethasone; TNF, tumor necrosis factor; IL, interleukin; CIA, collagen-induced arthritis; DRN, drug bound to receptor in the nucleus.
(DEX) into the disease progression model by assuming that DEX binds to the same receptor as CST and that both ligand-receptor complexes mediate the observed responses. Time courses of DEX effects and model fittings are presented for several disease progression markers: tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, GR mRNA, plasma CST, paw edema, and BMD for various dose regimens. Simulations indicating optimal dosing in rats for protecting BMD, while affecting cytokine expression and paw edema, are presented. The combination of mechanistic disease progression modeling with pharmacodynamic DEX effects provides a systems approach to resolving the role of CS treatment on cytokine suppression and subsequent cytokine effects on paw swelling and bone mineral density, and it suggests how optimal doses or combination therapies may be designed using knowledge of cytokine suppression and relevant contributions to disease endpoints.

Materials and Methods

Care of animals, induction of arthritis, measurements of paw edema, TNF-α, IL-1β, IL-6 mRNA, GR mRNA, CST, and BMD, and other details are presented in Earp et al. (2008).

Experimental Design

Dynamics of Cytokine mRNA, GR mRNA, and CST: Low and High Single-Dose Dexamethasone. On study day 0, 144 male Lewis rats were induced with CIA. Of these 144 rats, 78 rats developed edema in both paws. Animal body weights and paw edema were monitored on study days 0, 7, 9, 12, 15, 17, 19, 21, and at time of sacrifice. Two groups of 39 rats received either a low dose (0.225 mg/kg) or high dose (2.25 mg/kg) of s.c. dexamethasone on day 21. Four to five animals in each group were sacrificed at 1, 2, 4, 6, 8, 12, 24, 36, and 48 h after dose. Animals were sacrificed by aortal exsanguinations, and blood was collected in syringes containing sufficient EDTA to yield 1.5 mg/ml (4 mM) final concentration (Samtani and Jusko, 2005). Samples were centrifuged at 1800g for 10 min at 4°C. Plasma was collected and stored at −80°C. Skin was removed from hind paws, and the paws were excised above the ankle and flash-frozen in liquid nitrogen before storage at −80°C.

Bone Mineral Density Dynamics: 7-Day Multiple DEX Administration. An additional eight healthy rats and six rats induced with arthritis were scanned with the PIXImus II instrument (GE Healthcare, Chalfont St. Giles, UK) to assess changes in bone mineral density in the femur, paw, and lumbar regions after 7-day DEX administration. Healthy rats were divided into two groups of four and dosed once daily for 7 days with either 0.045 or 0.225 mg/kg s.c. DEX starting on day 21. The six arthritic rats were dosed once daily for 7 days with 0.225 mg/kg s.c. DEX starting on day 21. Healthy animals were scanned for the femur, lumbar, and paw regions on days 1, 8, 16, 20, 22, 24, 26, 30, and 33. Arthritic animals were scanned for the femur, lumbar, and paw regions on days 9, 12, 15, 20, 22, 24, 26, 30, and 33.

Paw and body weight data and tissues from preliminary pilot studies in our laboratory and from Earp et al. (2008) were also included in the model development. In total, 162 healthy and arthritic rats were used for all paw mRNA measurements. From this total, 220, 214, and 212 data points were used for TNF-α, IL-1β, and IL-6 mRNA (one to two paws were used per rat). Plasma corticosterone was assayed from 177 rats (177 data points), and paw edema was determined from 314 rats (4340 data points; 13 points per rat). Bone mineral density was assayed from 46 animals, and 1464 data points in total from five different scanned regions were modeled.

Pharmacodynamic Model of Dexamethasone Effects in Rat Arthritis

Figure 1 shows a general schematic for the entire arthritis progression model with DEX binding to GR. The structure of the disease progression model and relevant feedback by CST is presented in Earp et al. (2008). In general, all equations for cytokine mRNA, GR

![Fig. 1. Model schematic for corticosteroid and cytokine inter-regulation during arthritis progression. Parameters and symbols are explained in the text and tables. Lines with arrows indicate conversion to or turnover of the indicated responses. Lines ending in closed circles indicate an effect is being exerted by the connected factors.](image-url)
mRNA, paw edema, and BMD remain unchanged when integrating dexamethasone effects into the model, as it is the CS bound to receptor complex in the nucleus (DRN) that exerts the effect of both DEX and CST. In this model, equal concentrations of corticosterone bound to GR in the nucleus and of DEX bound to GR in the nucleus are treated as the same effectors on cytokine mRNA, GR mRNA, and CST. Equations that govern this binding and distribution of drug bound to GR in the nucleus are modified in the presence of DEX.

**Corticosterone and GR Dynamics.** The modified differential equation and initial condition describing concentrations of free GR in the presence of both CST and DEX is

\[
\frac{dGR}{dt} = k_{syn, GR} \times GR_{mRNA} - k_{dgr, GR} \times GR - k_{on, C} \times RF
\times GR + k_{on, D} \times DEX \times GR + k_{RE, C} \times RF \times DRN_C
\]

where \( k_{syn, GR} \) is the first-order synthesis rate of GR mRNA, and \( k_{dgr, GR} \) is the first-order loss rate constant. The parameters \( k_{RE, C} \) and \( k_{on, D} \) describe the rates that corticosterone bound to receptor and dexamethasone bound to receptor return from the nucleus. The parameter RF is the fraction of GR that returns intact and active. The values for \( k_{dgr, GR} \) and RF were fixed from previous studies (Hazra et al., 2007a,b, 2008). The second-order binding rate constant for CST was defined as

\[
k_{on, C} = \frac{k_{on, C}}{GC_{cyst}} \times \frac{1 \text{ nmol}}{346.47 \text{ ng}} \times \frac{6.59 \text{ nM}}{18.64 \text{ nM}}
\]

where 346.47 g is the molecular mass of CST, and 18.64 nM is the literature-reported \( K_D \) value for CST (Buchwald, 2008). The parameter \( f_{OC} \cdot k_{on, C} \) is the fraction of drug in plasma that is capable of binding in tissue. The second-order binding rate constant for DEX was defined as

\[
k_{on, D} = \frac{k_{on, D}}{GC_{cyst}} \times \frac{1 \text{ nmol}}{392.47 \text{ ng}} \times \frac{6.59 \text{ nM}}{18.64 \text{ nM}} \times k_{on, C}
\]

where 392.47 g is the molecular mass of DEX, and 6.59 nM is the literature-reported \( K_D \) value for DEX (Buchwald, 2008). The model assumes that CST equilibrium between vasculature and tissue is immediate. Bound CST-receptor complex in the cytosol is

\[
\frac{dDR_{CST}}{dt} = k_{on, C} \times CST \times GR - k_T \times DR_{CST}, \quad DR_{C}(0) = DR_{C0}
\]

and bound DEX-GR complex in the cytosol is

\[
\frac{dDR_{DEX}}{dt} = k_{on, D} \times DEX \times GR - k_T \times DR_{DEX}, \quad DR_{D}(0) = 0
\]

The modified differential equation and initial condition describing concentrations of bound CST-GR complex to the nucleus is

\[
\frac{dDRN_C}{dt} = k_t \times DR_{CST} - k_{RE, C} \times DRN_C, \quad DRN_{C}(0) = DR_{C0}
\]

where \( k_T \) is the first-order rate constant for translocation of bound CST-GR complex to the nucleus and is reported previously (Hazra et al., 2007a,b, 2008). Concentrations of bound DEX-GR complex in the nucleus are

\[
\frac{dDRN_D}{dt} = k_t \times DR_{DEX} - k_{RE, D} \times DRN_D, \quad DRN_{D}(0) = 0
\]

where \( K_D \) is the second-order dissociation constant for CST-GR complex to the nucleus and is reported previously (Hazra et al., 2007a,b, 2008). Concentrations of bound CST-GR complex in the nucleus are

\[
DR_{CST} = k_{on, C} \times CST \times GR - k_T \times DR_{CST}, \quad DR_{C}(0) = DR_{C0}
\]

where \( DR_{C0} = DR_{C0}(0) \) is the steadystate concentration of CST-GR complex to the nucleus and is reported previously (Hazra et al., 2007a,b, 2008). Concentrations of bound DEX-GR complex in the nucleus are

\[
DR_{DEX} = k_{on, D} \times DEX \times GR - k_T \times DR_{DEX}, \quad DR_{D}(0) = 0
\]

The modified differential equation and initial condition describing concentrations of free GR in the presence of both CST and DEX is

\[
\frac{dGR}{dt} = k_{syn, GR} \times GR_{mRNA} - k_{dgr, GR} \times GR - k_{on, C} \times RF
\times GR + k_{on, D} \times DEX \times GR + k_{RE, C} \times RF \times DRN_C
\]

where \( k_{syn, GR} \) is the first-order synthesis rate of GR mRNA, and \( k_{dgr, GR} \) is the first-order loss rate constant. The parameters \( k_{RE, C} \) and \( k_{on, D} \) describe the rates that corticosterone bound to receptor and dexamethasone bound to receptor return from the nucleus. The parameter RF is the fraction of GR that returns intact and active. The values for \( k_{dgr, GR} \) and RF were fixed from previous studies (Hazra et al., 2007a,b, 2008). The second-order binding rate constant for CST was defined as

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where 392.47 g is the molecular mass of DEX, and 6.59 nM is the literature-reported \( K_D \) value for DEX (Buchwald, 2008). The model assumes that CST equilibrium between vasculature and tissue is immediate. Bound CST-receptor complex in the cytosol is

\[
\frac{dDR_{CST}}{dt} = k_{on, C} \times CST \times GR - k_T \times DR_{CST}, \quad DR_{C}(0) = DR_{C0}
\]

where \( DR_{C0} = DR_{C0}(0) \) is the steadystate concentration of CST-GR complex to the nucleus and is reported previously (Hazra et al., 2007a,b, 2008). Concentrations of bound DEX-GR complex in the nucleus are

\[
\frac{dDRN_C}{dt} = k_t \times DR_{CST} - k_{RE, C} \times DRN_C, \quad DRN_{C}(0) = DR_{C0}
\]

where \( k_T \) is the first-order rate constant for translocation of bound CST-GR complex to the nucleus and is reported previously (Hazra et al., 2007a,b, 2008). Concentrations of bound DEX-GR complex in the nucleus are

\[
\frac{dDRN_D}{dt} = k_t \times DR_{DEX} - k_{RE, D} \times DRN_D, \quad DRN_{D}(0) = 0
\]

The value of \( DR_{C0} \) for the presence of the two CS is then calculated as the sum of concentrations of each drug bound to receptor in the nucleus: \( DR_{C0} = DR_{C0}(CST) + DR_{C0}(DEX) \). The parameters \( R_0_{GR} \), \( R_0_{DR} \), and \( R_0_{DRN} \) are defined based on the steady-state baseline conditions as described in Earp et al. (2008).

Production of plasma CST concentrations is up-regulated by inflammation, and it is correlated with proinflammatory cytokines and inhibited by DEX-GR complex in the nucleus.

\[
\frac{dCST}{dt} = k_{on, C} \times CST \times GR - k_T \times DR_{CST} - k_{dgr, GR} \times CST - k_{dgr, GR} \times CST
\]

where \( k_{on, C} \) is the first-order rate constant for the production of CST, and \( IC_{50, CST} \) is the amount of \( DR_{C0} \) required to inhibit production of CST 50%. The variable \( B_{CTT} \) is the influence of proinflammatory cytokines on CST up-regulation defined as

\[
B_{CTT} = \beta_1 \times (TNF_{mRNA} - R_{0,TNF}) + \beta_2 \times (IL1_{mRNA} - R_{0,IL1})
\]
where $\beta_1$ and $\beta_2$ are the intrinsic activities of each individual cytokine on CST up-regulation.

Dynamics of Paw Edema and Bone Mineral Density. Pharmacodynamic models of drug effect for paw edema and bone mineral density are as presented in Earp et al. (2008).

Disease-State Pharmacokinetics of Dexamethasone. The pharmacokinetic model for dexamethasone concentrations after 0.225 and 2.25 mg/kg s.c. doses in healthy and arthritic animals was applied to describe the kinetics of s.c. dexamethasone. Equations and initial conditions describing the amounts of DEX in each compartment are

$$\frac{dA_{\text{Abs}}}{dt} = -k_a \times A_{\text{Abs}}, \quad A_{\text{Abs}}(0) = \text{Dose} \times F$$

$$\frac{dA_{\text{pl}}}{dt} = k_a \times A_{\text{Abs}} + \frac{\text{CL}_{i,j}}{\sqrt{\text{VT}}} \times A_T - \left( \frac{\text{CL}_{i,j}}{\sqrt{\text{Vp}}} - \frac{\text{CL}_{i,j}}{\sqrt{\text{VT}}} \right) \times A_{\text{pl}}, \quad A_{\text{pl}}(0) = 0$$

$$\frac{dA_T}{dt} = \frac{\text{CL}_{i,j}}{\sqrt{\text{Vp}}} \times A_{\text{pl}} - \frac{\text{CL}_{i,j}}{\sqrt{\text{VT}}} \times A_T, \quad A_T(0) = 0$$

where $A_{\text{Abs}}$ indicates the amount at the absorption site, $A_{\text{pl}}$ is the amount of DEX in plasma, $A_T$ is the amount in peripheral tissues, $k_a$ is the first-order absorption rate constant (5.78 h$^{-1}$), CL is the plasma clearance (1.05 and 1.19 l h$^{-1}$ kg$^{-1}$ for healthy and arthritic rats), $\text{CL}_{i,j}$ is the intercompartmental clearance (7.20 l h$^{-1}$ kg$^{-1}$), $\text{Vp}$ is the central volume of distribution (3.41 l), $\text{VT}$ is the peripheral volume (1.44 l), and F is the i.m. bioavailability (0.86). Concentrations of DEX in plasma were generated from $C_{\text{pl}} = A_{\text{Abs}}/\sqrt{\text{Vp}}$.

Model Fitting and Nonlinear Regression Analysis

The pharmacodynamic model was constructed in two phases with the disease progression data and drug effect data modeled simultaneously. The first model fitting phase included both disease progression (Earp et al., 2008) and drug effect data on cytokine mRNA, GR mRNA, and plasma CST. The relevant parameters were obtained and then fixed for the second model-fitting phase where paw edema and BMD data from both Earp et al. (2008) and this study were modeled simultaneously. Drug effect-related data, parameters, and model-fitted curves are presented here. Modeling fittings were performed using S-ADAPT (Biomedical Simulations Resource, University of Southern California, San Diego, CA) as described in Earp et al. (2008). Model simulations were done using Berkeley Madonna Software (University of California, Berkeley, CA) implementing the Rosenbrock (stiff) method for solving differential equations. Simulated values of the BMD at steady state were used to determine the dose at which the maximal steady-state response after treatment was observed. The initial lower and upper bounds of dose in the numerical bisection algorithm were 0 and 2.25 mg/kg once daily. These bounds were narrowed until there was less than a 0.001-mg/kg difference between the upper and lower bounds while keeping the maximal response between these two limits. The optimal dose was reported to three significant digits.

Results

The pharmacokinetics of DEX after i.m. administration was studied previously in healthy and arthritic rats (Fig. 2). A pilot study in healthy rats showed concentrations after s.c. and i.m. dosing to be identical. Plasma DEX concentrations were measured in samples collected in this study, and they were in agreement with those from i.m. dosing. The same kinetic model for i.m. administration was applied for healthy and arthritic rats after s.c. DEX dosing.

Profiles for cytokine mRNA expression after single s.c. doses of 0.225 or 2.25 mg/kg DEX are shown in Fig. 3. TNF-$\alpha$ mRNA shown in Fig. 3A had an unexpected time course after DEX dosing. There was an abrupt rise followed by a drop, returning to a rise above the natural disease progression for both doses, although the inhibition by DEX was maintained longer with the higher dose. The IL-1$\beta$ mRNA was initially inhibited abruptly and almost completely after both DEX doses; however, the inhibition was maintained near 100% for a longer time with the higher dose. Interleukin-6 was inhibited very rapidly and completely in a similar manner. No difference in drug effects between low and high doses was observed with IL-6, indicating a high degree of sensitivity to CS. Interestingly TNF-$\alpha$ was the only cytokine with increased mRNA expression after DEX. Relevant parameter estimates for drug effects on cytokine mRNA are presented in Table 1. The IC$_{50}$ values for these cytokines were in agreement with the observed responses. The lowest value of 4.5
nM was for interleukin-6, indicating the greatest sensitivity to CS effect, followed by IC$_{50}$,IL-1β of 32.6 nM, and TNF-α at 550 nM. The release constant for DEX-GR complex from the nucleus $k_{r,1}$ (hours$^{-1}$) = 0.0498] was also much lower than that of CST-GR complex $k_{r,1}$ (hours$^{-1}$) = 85.04]. The remaining disease progression model and parameters are presented in Earp et al. (2008).

Dexamethasone effects on GR mRNA and endogenous CST are shown in Fig. 4, A and B, after single s.c. doses of 0.225 or 2.25 mg/kg DEX, and they are compared with no drug treatment. For the low dose, there seemed to be a quick drop followed by a rebound in GR mRNA, whereas the high dose showed a sharp drop that remained below disease progression values throughout 48 h after dose. Data for CST are shown in Fig. 4B. No differences with dose were observed because CST was suppressed very rapidly and nearly completely. Drug effect parameters for GR mRNA and CST are presented in Table 1. The IC$_{50}$ of GR was fairly high, which accounts for the low dose having almost no response, whereas the high dose produced a sharp drop, necessitating the high value of the Hill coefficient.

Paw edema responses in rats with CIA after single 0.225 and 2.25 mg/kg as well as seven once-daily multiple doses of 0.225 mg/kg DEX are shown in Fig. 5. For all doses, edema dropped rapidly. However, multiple dosing was required to suppress paw size close to the healthy baseline. Returns to disease state values were not observed during the first 48 h after acute single doses. Instead, this return was drawn out over 100 to 300 h as indicated by the chronic dosing paw edema data. Parameter estimates for paw edema are present in Earp et al. (2008) along with model equations. Model fittings captured the profiles fairly well, although there was a general overprediction of suppression with the low dose, and the extent to which the multiple dosing would reduce the overall paw edema was also slightly overpredicted.

Figure 6 shows bone mineral density progression in the femur and lumbar regions of healthy and arthritic rats after seven once-daily multiple dosing of either 0.045 mg/kg in healthy rats or 0.225 mg/kg DEX in healthy and arthritic rats. Early in time, there is an increase in BMD from natural growth of the 6- to 9-week-old rats. After the first dose at 504 h in the arthritic group, there was an initial increase in BMD followed by a further decline. In healthy rats, there was a delay of approximately 100 h before the effects of DEX were observed on BMD. Unlike for paw edema, DEX is known to regulate other factors besides cytokine mRNA in bone turnover. The model incorporated DEX effects through both inhibition of osteoblast production and reduced proinflammatory cytokine mRNA production. The relevant IC$_{50}$ value for CS inhibition of osteoblast production is listed in Table 1, with a value of 984 nM. In the lumbar region, effects of arthritis were not immediately apparent, although it was observed that systemic CS did reduce BMD later in time. Model fittings of BMD in general captured the data well.

Simulated concentrations of DR$_{N}$ for all DEX dosing regimens are shown in Fig. 7. These simulations are particularly useful when comparing the IC$_{50}$ values for DR$_{N}$ for each type of response. Figure 8 shows simulations of total femur BMD, lumbar BMD, cytokine mRNAs, DR$_{N}$, and paw edema after chronic treatment with 0, 0.0113, or 0.225 mg/kg DEX once

### Table 1

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<th>Parameter (Units)</th>
<th>Description</th>
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$^a$ S-ADAPT Software was unable to generate percentage coefficient of variation.

$^b$ Parameter value was fixed.

*Fig. 4.* Time courses for GR mRNA in paw (A) and plasma corticosterone (B) after 0.225 and 2.25 mg/kg s.c. DEX and no drug treatment. Closed circles depict mRNA after the single high dose. Open triangles depict mRNA after the single low dose. Black arrows indicate the time of dosing (504 h post-CIA induction). Open squares show the natural disease progression time course of GR mRNA. Data and model fittings are as described in Fig. 3.
daily. The 0.0113-mg/kg dose was determined through analysis of steady-state equations to be the optimal chronic DEX dose for preventing BMD loss. Higher DRN concentrations than produced by 0.0113 mg/kg meant that inhibition of bone production would cause a greater loss in BMD, whereas lower concentrations of DRN would yield less proinflammatory cytokine suppression and result in stimulation of bone loss. The 0.225-mg/kg dose was initially chosen to produce concentrations in the rat similar to those in low dose clinical regimens. If 0.225 mg/kg in the rat is equivalent to the minimal clinically effective dose, then the model would indicate this dosing scenario to greatly reduce bone mineral density (Fig. 8, A and B). Wider gray lines in these figures indicate oscillations between peak and trough responses from serial dosing every 24 h. Interesting was that the “optimal dose” produced a general increase in BMD despite an increase in TNF-α mRNA expression, indicating that IL-1β and IL-6 would more strongly regulate BMD. Despite the reduction in IL-1β and IL-6 mRNA, paw edema was not dramatically reduced, highlighting the effect of TNF-α on edema.

Discussion

Developing mechanistic models of drug effects in disease states requires understanding of both disease progression and how the drug acts on intermediary factors. We examined CIA progression in the rat and subsequent effects of DEX on the relevant factors driving edema and bone turnover. Endogenous CST plays a role in mediating cytokine turnover in disease progression (Turnbull and Rivier, 1999; Neeck et al., 2002). Therefore, effects of DEX and other CS may be included with minimal modifications of the disease progression model. Only the drug kinetics and drug-receptor binding need definition. Our experimental design effectively integrates DEX pharmacodynamics with disease progression to better resolve parameters of both components.

Pharmacodynamic data for DEX are critical for understanding the interrelationships between cytokines, GR mRNA, and CST. As the disease progresses, immune factors such as chemo/cytokines turn over quickly. Corticosterone concentrations and GR mRNA are up-regulated slowly so that cytokines remain in balance and no abrupt inhibition or
changes are observed in the natural disease progression. However, when DEX is administered and concentrations of DRN spike, rapid changes occur in all relevant profiles, revealing rate-limiting steps, rates of loss and production, and edema and BMD responses to different concentrations of cytokines. This modeling effort not only extracts quantitative biological relationships concerning cytokine effects on paw edema and BMD but also yields implications about the role of corticosteroids and other therapies that target cytokines in inflammatory arthritis.

The time course of TNF-α mRNA after acute 0.225 and 2.25 mg/kg s.c. DEX was unusual because of the increase in TNF-α expression both early and later in time. Interestingly, IL-6 was shown to inhibit the expression of TNF-α (Schindler et al., 1990). With the rapid and near complete drop in IL-6 mRNA production after dose, it is possible that if IL-6 consistently inhibited TNF-α production, then TNF-α mRNA would increase when IL-6 concentrations fell. Based on the in vitro evidence for this suppression, the maximal suppression of TNF-α mRNA was fixed at 30% inhibition (Schindler et al., 1990). The model then captured both this rise and fall and rise again in TNF-α mRNA response.

That the expression of IL-6 mRNA was highly sensitive to DEX indicates that the decline observed in IL-6 mRNA was related to CST. With increased CST and GR mRNA, DRN was increased sufficiently to cause a drop in IL-6 but not in the other measured cytokine mRNA. Although this explains in part the decline of IL-6 mRNA, it could not be the entire reason. If DRN increased rapidly as in the case of CS dosing, CST would be inhibited almost completely and for a prolonged time, such that when DRN returned to normal and CST remained suppressed, the IL-6 mRNA production would overshoot the measured response. The “remission” compartment presented in Earp et al. (2008) helped capture this decline. It is possible that as the disease develops into a more chronic state, there is a shift from the innate to a more humoral immune response, effectively altering concentrations of proinflammatory cytokines.

Turnover of GR mRNA after DEX behaved differently than the cytokines. There was an abrupt decline followed by a rebound to values higher than found in the natural disease progression with the low dose. If TNF-α had a larger contribution to GR mRNA up-regulation, then it is possible that this rebound would have been observed. This would have also been seen for the higher dose. A time delay was necessary to account for the slow rate of disease progression that did not plateau as quickly as cytokine mRNAs, whereas a fast drop was observed in response to DEX. Transit compartments accounted for the delayed response to cytokines while allow-
ing the equation describing GR to have a high rate of turnover to reflect rapid drug effects.

Binding of DEX to GR was modeled using literature reported $K_D$ values to adjust the binding of CST in the presence of DEX in such a way that only $f_f$ $k_{on,C}$ was fitted for both drugs. This parameter corrects for both the fraction of drug that equilibrates from plasma into tissue and the free fraction able to bind receptor. Distribution is assumed to be instantaneous. The release rate constants ($k_{RE,C}, k_{RE,D}$) from the nucleus were reasonably estimated compared with the $k_{RE}$ value for MPL ($k_{RE,M} = 1.31$ h$^{-1}$) because CST is less potent than MPL, but DEX is more potent. The release constant for DEX-GR complex from the nucleus was also much lower than that of CST-GR complex and MPL-GR complex. If both molecules exert their effects to the same extent through GR in the nucleus, then DEX would need to remain bound in the nucleus for a longer duration to yield the differences in observed inhibition of cytokine mRNA for CST during disease progression versus DEX concentrations after dosing. Interestingly, the profile for $D_{RN}$ after chronic dosing of 0.045 mg/kg DEX (Fig. 6) quickly approached the steady-state $D_{RN}$ as exhibited for 0.225 mg/kg DEX. This meant that the single 2.25 mg/kg and 7-day 0.225 multiple doses of DEX were probably exerting near maximal responses. This steady state was limited by the concentrations of free GR receptor. The estimate for synthesis of GR in inflamed tissue was 0.1054 h$^{-1}$ compared with 0.54 h$^{-1}$ in liver tissue reported previously (Schindler et al., 1990; Ramakrishnan et al., 2002; Hazra et al., 2007b, 2008).

Because DEX affects cytokine mRNA expression and not edema directly, it is the inhibition of cytokines that governs the decline in paw edema. In general, the model captured that data well, with modest overprediction for the 0.225 mg/kg acute and chronic dosing. This overprediction may be due in part to the linear additive relationship between cytokine mRNA and paw edema. If the relationship between cytokines and edema is receptor driven, then a Hill-type effect relationship may be more appropriate. It is also possible that because there are other processes that contribute to edema not included in the model, their effects on up-regulating paw edema are missed. However, altering each signaling pathway alone and simultaneously in different combinations

Fig. 8. Simulated profiles of femur BMD (A), lumbar BMD (B), TNF-α mRNA (C), IL-1β mRNA (D), IL-6 mRNA (E), $D_{RN}$ (F), and paw edema (G) after no drug treatment (solid black lines), chronic 0.0113 mg/kg DEX daily (gray lines), and 0.225 mg/kg DEX daily (black long-short dashed lines) in arthritic rats. Short dashed lines depict BMD responses in healthy rats. DEX dosing begins 504 h after induction and is repeated every 24 h for the remainder of the simulation.
while measuring relevant paw edema is impractical; thus, the true effect of cytokines on edema may be mis-specified. For the key inflammatory signaling pathways that were measured, this model captured the data well, and linear stimulatory parameters reflect the relative contributions of each cytokine to paw edema.

The effect of DEX on bone was observed by two different responses. 1) An increase in BMD was observed almost immediately in arthritic animals as concentrations of proinflammatory cytokines fell. 2) Effects of DEX on osteoblast apoptosis/reduced activity resulted in decreased BMD in all scanned regions of both healthy and arthritic animals approximately 100 h (5 days) after the first dose. Higher doses of CS overpowered the anti-inflammatory effects, yielding a reduced BMD. Figure 8 indicates that much lower doses of DEX were sufficient to reduce cytokines IL-1β and IL-6 mRNA, increasing BMD and mitigating adverse effects. The cytokine mRNA with the least effect on BMD was TNF-α, which had the highest IC₅₀ value (550 nM). The cytokine with the lowest IC₅₀ value (4.5 nM), IL-6, had the greatest contribution to BMD loss. Owing to the cytokine most sensitive to DR₅ contributed the most to reducing BMD density provided a potential explanation for why recent studies involving low dose CS were able to halt radiographic damage to joints (Svensson et al., 2005; Wassenberg et al., 2005; Da Silva et al., 2006).

The doses of 0.225 and 2.25 mg/kg were chosen to produce DEX concentrations in rat plasma similar to the lowest and highest exposures in humans. If these are relevant clinical doses based on pharmacokinetics, free fraction of drug, and receptor binding constants, then Fig. 8 would suggest that the minimally effective doses (0.225 mg/kg in the rat) are 20 times in excess of the optimal dose. Concentrations at higher doses of DEX will produce effects on bone loss that will dominate the protective effects. Owing to the lower binding affinity and potentially reduced kₒ value for prednisolone, concentrations of DR₅ may approach those of this lower optimal dose and explain why recent studies with low-dose prednisolone have seemed protective for BMD (Buchwald, 2008). In addition, the model suggests that because lower doses of CS should be given and these doses may be insufficient to suppress TNF-α, a second agent given to either reduce concentrations of TNF-α or inhibit TNF-α effects on inflammation would be beneficial to both edema and BMD responses.

Owing to the extensive clinical history of corticosteroid use, their pharmacokinetics are well established as are receptor binding constants making this modeling paradigm potentially useful for translation to the clinic. It is advantageous that the model processes are based on known physiology of rheumatoid arthritis and that the major factors understood to control inflammation can be related to each other to describe disease progression and drug effects on both the molecular and symptomatic aspects of chronic autoimmune arthritis.

References


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