Journal of Pharmacokinetics and Biopharmaceutics, Vol. 22, No. 6, 1994

Pharmacodynamic Model for Joint Exogenous and Endogenous Corticosteroid Suppression of Lymphocyte Trafficking

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Received August 2, 1994—Final January 18, 1995

The circadian pattern of the immune system correlates with that of circulating T-helper cells and inversely with cortisol concentrations. Corticosteroids, both endogenous and exogenous, cause lymphocyte dimunition in blood by retention of cells in the lymphatic circulation. A physiologic pharmacodynamic model was developed to describe changes in circulating lymphocytes as a function of both endogenous cortisol and methylprednisolone concentrations. The model was applied to T-helper and T-suppressor cell data collected from six asthmatic men during baseline, after single-dose, and after 6 days of 20 mg daily methylprednisolone. The model described all phases of the study well. Baseline circadian rhythm of lymphocytes was related to cortisol concentrations. Multiple-dosing of methylprednisolone caused apparent tolerance and decreased the sensitivity of lymphocytes to corticosteroids by 116% and markedly reduced endogenous cortisol concentrations. A 60% increase in circulating T-helper cells was observed which could be accounted for by dual changes in receptor sensitivity and endogenous cortisol.

KEY WORDS: pharmacodynamics; T-helper cells; T-suppressor cells; CD4+ lymphocytes; CD8+ lymphocytes; corticosteroids; methylprednisolone; cortisol; tolerance.

INTRODUCTION

The effects of synthetic exogenous corticosteroids on lymphocyte trafficking patterns are well studied. Corticosteroids cause efflux of some

Supported by National Institutes of Health Grant 24211 from the National Institute of General Medical Sciences and by the Sandoz Pharmaceutical Research Fellowship in Immunology Therapeutics from the American College of Clinical Pharmacy for Dr. Milad.

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0090-466X/94/1200-0469$07.00/0

lymphocytes from the vasculature and retention of these cells in the lymphatic circulation (1). The mechanism by which corticosteroids cause such movements may be related to cell adhesion molecules (2). Due to the relative differences in concentrations and potency, the effect of endogenous cortisol on lymphocyte trafficking is less obvious than that from more potent synthetic corticosteroids. However, the circadian pattern of T-helper cells in blood correlates inversely to that of endogenous cortisol (3–6). This suggests that the circadian pattern of T-helper cells is influenced by cortisol.

Mathematical models used to describe the pattern of T-helper cell redistribution after single-dose corticosteroid therapy have generally worked well, however, they do not take into account endogenous cortisol concentrations and fail when lymphocyte numbers rise above those seen during baseline. The model proposed here, in part, describes the circadian pattern of lymphocytes as a function of endogenous cortisol. The differential equations which were developed describe changes in lymphocyte numbers during baseline and after methylprednisolone employing both cortisol and methylprednisolone concentrations.

METHODS

The pharmacodynamic model was developed for application to a recent study. Briefly, six asthmatic male volunteers, average age 24 years (range 19–34) and within 20% of ideal body weight, were enrolled. All subjects had mild asthma with peak expiratory flow rates greater than 80% of predicted, and were not exposed to exogenous corticosteroids within the previous 30 days.

Each subject underwent three phases in consecutive order. Phase 1 employed sampling every 2 hr for 24 hr to assess baseline patterns of cortisol and T-helper and T-suppressor cells.

Thirteen days later, Phase 2 started at 8 AM when 20 mg of methylprednisolone was administered as the sodium succinate ester. Blood samples were collected for determination of methylprednisolone and cortisol plasma concentrations at 0, 0.25, 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28, and 32 hr. Blood samples were collected for lymphocyte determinations at 0, 1, 2, 4, 8, 12, 16, 20, 24, and 32 hr.

Phase 3 started 48 hr after the initiation of Phase 2. Subjects received a daily oral dose of 20 mg methylprednisolone for 5 days. On Day 6, each subject repeated a similar protocol as described in Phase 2. Therefore, Phase 3 represents data following 6 consecutive days of exposure to 20 mg of methylprednisolone.

Cortisol and methylprednisolone concentrations were measured by the HPLC method of Ebling *et al.* (7). A modified commercial RIA kit (Coat-A-Count, Cortisol, Diagnostic Products Corp., Los Angeles, CA) was used to measure cortisol concentrations of less than 10 ng/ml and when cortisol and methylprednisolone concentrations were less than the minimal detectable level by HPLC (10 ng/ml).

T-helper and T-suppressor cell counts were determined by reacting the whole blood sample with anti-CD3, anti-CD4 and anti-CD8 antibodies, and subsequently measuring fluorescence by flow cytometry (FACScan, Becton-Dickinson, Mountain View, CA). T-helper cells were immunoreactive as CD3+, CD4+, and CD8−; T-suppressor cells were CD3+, CD4−, and CD8+.

The observed data were fitted to the model using the PCNONLIN computer program (SCI Software Inc., Lexington, KY). Weights equal to 1 were used in fitting all pharmacodynamic data. All three phases were fitted simultaneously. Statistical analysis included a paired Student t test to compare parameters of Phases 1 and 2 to those of Phase 3. All results were considered significant at the $p < 0.05$ level.

PHARMACODYNAMIC MODEL

The change in lymphocyte concentrations in blood versus time is described by Eq. (1) (8). Theoretically, cells traffic between the blood and extravascular compartments. R_{in} represents the zero-order rate constant which describes cell return to blood when corticosteroids are absent from the system. The first-order rate constant, k_{TH}, reflects lymphocyte egress from the vasculature.

$$\frac{dTH}{dt} = R_{in} \cdot I(t) - k_{TH} \cdot TH \tag{1}$$

The inhibition function, $I(t)$ is

$$I(t) = 1 - \frac{C_{mp} \cdot \xi + C_{cort}}{IC_{50} + C_{mp} \cdot \xi + C_{cort}} \tag{2}$$

The inhibition term employs cortisol, C_{cort}, and methylprednisolone concentrations, C_{mp}, and a constant, IC_{50}. The IC_{50} term is defined as the concentration of cortisol which reduces R_{in} by 50%. A ratio term, ξ, accounts for the differences in potency between methylprednisolone and cortisol. Equation (2) assumes that the maximum effects of methylprednisolone and cortisol are equal (i.e., $I(t) \rightarrow 0$ at high values of C_{mp} or C_{cort}). The inhibition function $I(t)$ was derived from equations of Oosterhuis and Van Boxel (9) who

described two different agonists acting competitively at the same receptor site (see Appendix).

Log-linear regression of the data points collected during the first 4 hr of Phases 2 and 3 was used separately to determine k_{TH}. At high C_{mp} concentrations, $I(t)=0$ and Eq. (1) becomes $dTH/dt=-k_{TH}\cdot TH$. Thus the initial decline in log TH cell numbers versus time produces a slope of $-k_{TH}/2.3$. The k_{TH} used for Phase 1 was the average of the two k_{TH} values from Phase 1 and 2. Initial estimates for R_{in} were obtained by multiplying the largest value for cell numbers by the k_{TH} value. A published (10) methylprednisolone to cortisol potency ratio of 9 was multiplied by the IC_{50} values for T-helper cell inhibition by methylprednisolone (11), to obtain an initial estimate of the IC_{50} value in terms of cortisol concentrations; this IC_{50} value was 90 ng/ml. Changes in the intrinsic sensitivity (IC_{50}) of the T-helper cells and T-suppressor cells after multiple dosing were evaluated by allowing the IC_{50} value for Phases 1 and 2 to differ from Phase 3.

Methylprednisolone concentrations, C_{mp}, were described in the pharmacodynamic model using an equation for monoexponential elimination following first-order formation (k_f) of methylprednisolone from the succinate ester

$$C_{mp}=\frac{k_f\cdot \text{dose}}{V\cdot(k_f-CL/V)}\left(e^{-(CL/V)\cdot t}-e^{-k_f\cdot t}\right) \tag{3}$$

Fitted values of k_f, clearance, CL, and volume of distribution, V, were used for Phases 2 and 3 for each subject.

Cortisol concentrations, C_{cort}, were difficult to describe accurately because of the data pattern and need for a continuous function which could be used in a differential equation via Eq. (2). Therefore, C_{cort} was fitted separately for each phase using an n^{th} order polynomial equation of the form

$$C_{cort}=a+b\cdot t+c\cdot t^2+d\cdot t^3+\cdots+x\cdot t^n \tag{4}$$

where $a, b, c, d, \ldots x$ are fitted coefficients. Characterization of the lymphocyte data with the pharmacodynamic model in Fig. 1 thus entailed use of Eq. (1) with insertion of Eqs. (2)–(4). Least squares values of R_{in}, IC_{50} (cortisol) for single versus multiple doses, and ξ were then generated using the PCNONLIN program.

Goodness-of-fit was determined by assessing the absence of systemic deviations of residuals and using the Akaike Information Criterion in comparison of preliminary and final model fittings.

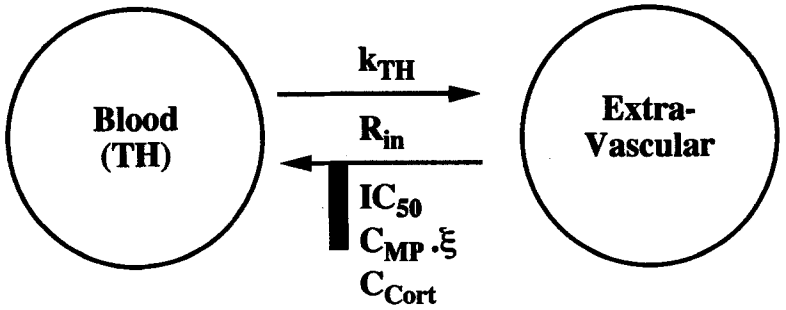

Fig. 1. The lymphocyte suppression model. The concentration of T-helper cells is represented by *TH*, k_{TH} is the first-order rate constant describing cell egress from the blood, R_{in} is a zero-order rate constant for cell return to the blood from extravascular sites, IC_{50} is the concentration of cortiocosteroid which inhibits R_{in} by 50%, C_{mp}, and C_{cort} are the plasma concentrations of methylprednisolone and cortisol, and ξ is the potency ratio of methylprednisolone:cortisol.

RESULTS

Figure 2 shows the observed methylprednisolone concentrations as a function of time for one subject and the least square curves fitted to Eq. (3). These data were appropriately described by Eq. (3). Mean values were $k_f = 6.99 \pm 3.3\ hr^{-1}$, $CL = 335 \pm 67$ ml/hr per kg, and $V = 1.19 \pm 0.11$ L/kg. No systematic changes in these parameters occurred on multiple dosing.

Figure 3 shows the observed cortisol concentrations and the fitted polynomials for a representative subject in all phases of the study. The baseline phase shows a normal circadian pattern of cortisol concentrations while the single dose of methylprednisolone caused acute suppression but a later return to baseline. Multiple dosing with methylprednisolone resulted in substantial adrenal suppression throughout the observation period. The polynomials used to describe cortisol concentrations closely simulated the actual concentrations observed during the study.

The observed and fitted T-helper cell numbers for one subject is shown in Fig. 4. All subjects had a normal baseline circadian pattern of these cells. The nadir occurred at 8 AM (Time 0 and 24 hr after dosing) and the acrophase was found at 12 to 4 AM (Time 16 to 20 hr). This pattern is opposite to that of cortisol concentrations during baseline (see Fig. 3).

After both single and mutliple dosing, the T-helper cells decreased to their nadir at approximately 8 hr with a return to baseline about 24 hr after the dose was given. During Phase 3, T-helper cell concentrations were 60% greater (compared to Phases 1 and 2) at Time 0, 24, and 32 hr in five of the six subjects. One subject showed no such rebound.

The concentrations of T-helper and T-suppressor cells during all three phases of the study were adequately described by the pharmacodynamic

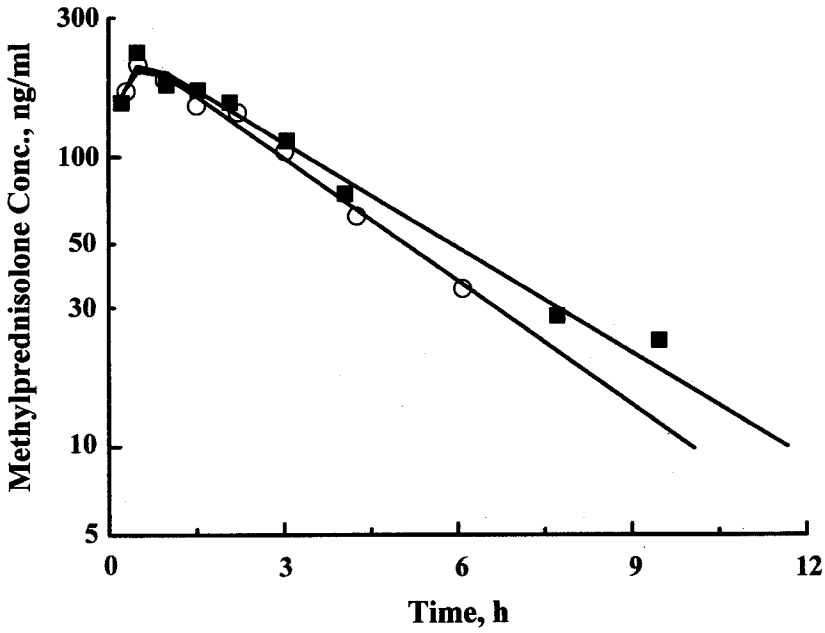

Fig. 2. Observed methylprednisolone concentrations after single dose (○), and after multiple dosing (■) of methylprednisolone in one subject. Curves are fitted to Eq. (3) using least squares regression.

model. The equations appropriately characterize the rebound of cells which occurs due to the sensitivity changes of lymphocyte recirculation and the suppression of cortisol after multiple dosing.

The fitted pharmacodynamic parameters ($\bar{x} \pm SD$) are shown in Table I. The cortisol IC_{50} value increased after multiple dosing from 66.3 ± 13.3 to 142.4 ± 60.4 ng/ml ($p < 0.05$) for T-helper cells and 56.0 ± 33.4 to 175.5 ± 75.6 ng/ml ($p < 0.05$) for T-suppressor cells. This represents decreases in sensitivity of 116 and 238%. The mean ξ found to account for the potency difference between cortisol and methylprednisolone was approximately 16 for T-helper cells and 11 for T-suppressor cells; these differ slightly from a previously reported potency ratios of 9 based on inhibition of *in vitro* lymphocyte proliferation (10). Model fitting of the data was inadequate when it was attempted to hold the IC_{50} values constant between single and mutliple dose phases.

DISCUSSION

The circadian rhythm of circulating lymphocytes has been shown to be related inversely to plasma cortisol concentrations (4–7) and may account

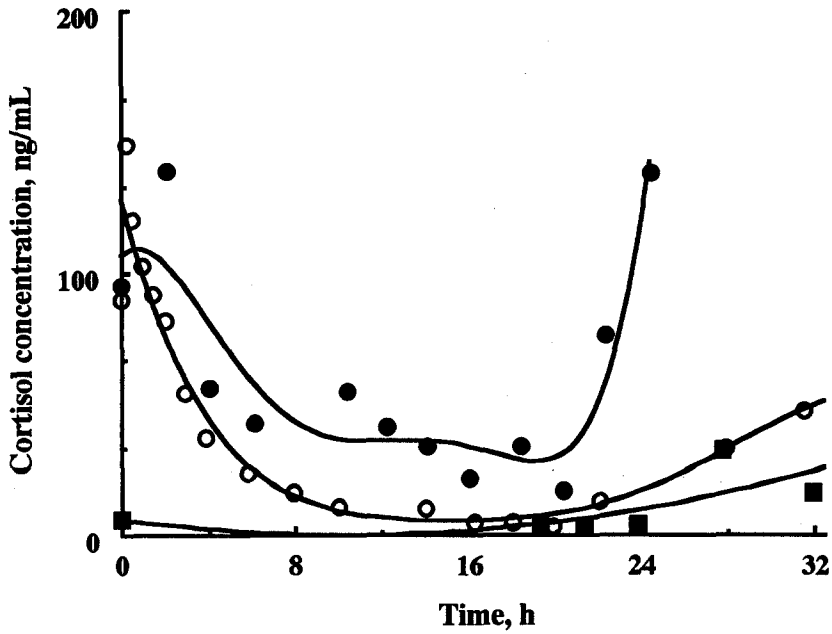

Fig. 3. Observed cortisol concentrations during baseline (●), after single dose (○), and after multiple-dosing (■) of methylprednisolone in one subject. Curves represent the following polynomials [Eq. (4)] used to describe the cortisol concentrations: Phase 1: $C_{cort} = 107 + 7.84t - 6.10t^2 + 0.789t^3 - 0.0399t^4 + 7.14 \cdot 10^{-4}t^5$ ($r^2 = 0.82$). Phase 2: $C_{cort} = 128 - 31.9t + 3.41t^2 - 0.186t^3 + 5.08 \cdot 10^{-3}t^4 - 5.29 \cdot 10^{-5}t^5$ ($r^2 = 0.93$). Phase 3: $C_{cort} = 6.32 - 1.03t + 0.0415t^2$ ($r^2 = 0.97$).

Table I. Pharmacodynamic Parameters ($\bar{x} \pm SD$) for Lymphocyte Suppression

	Baseline and single dose	Multiple dose
T-helper cells		
IC_{50}, ng/ml cortisol	66.3 ± 13.3	142.4 ± 60.4[b]
IC_{50}, ng/ml methylpred.[a]	4.95 ± 1.81	9.42 ± 1.44[b]
R_{in}, cells/mm^3 per hr	630 ± 138	
ξ	15.9 ± 8.6	
T-suppressor cells		
IC_{50}, ng/ml cortisol	56.0 ± 33.4	175.5 ± 75.6[b]
IC_{50}, ng/ml methylpred.[a]	6.53 ± 4.35	18.2 ± 4.87[b]
R_{in}, cells/mm^3 per hr	208 ± 105	
ξ	11.3 ± 8.4	

[a]Calculated from IC_{50} cortisol/ξ.
[b]$p < 0.05$.

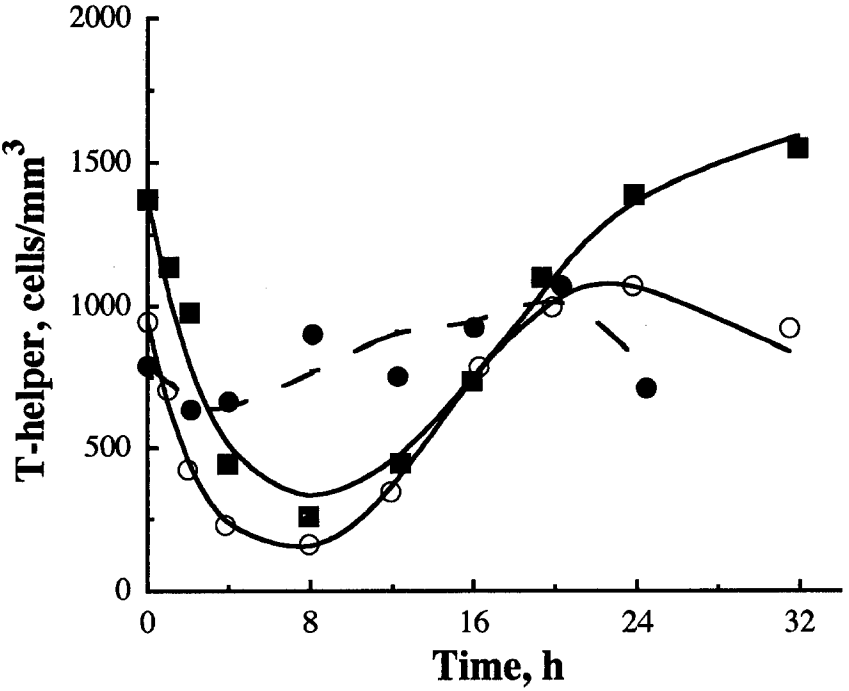

Fig. 4. Observed T-helper cell concentrations during baseline (●), after a single dose (○) and after multiple doses (■) of methylprednisolone in one subject. Curves are fitted to Eqs. (1)-(2) using least squares regression.

for circadian rhythms in immune function. Tests of cutaneous reactivity, the size of erythema and wheals after an injection of histamine or antigens, exhibit a similar circadian pattern as seen with T-helper cells (12). The same circadian pattern is seen in asthmatic patients. Seventy percent of deaths due to asthma occur at night, between 8 PM and 8 AM (13).

Acute asthmatic attacks have been related to peripheral concentrations of lymphocytes. Activated T-helper cells are found in the peripheral blood of patients who are experiencing an acute asthmatic attack (14). Furthermore, changes in peripheral activated T-helper cells have been correlated with changes in peak expiratory flows rates (15). Therapy and clinical improvement accompany a decreased number of activated T cells (14). Although the present study did not measure activated T cells, two T cell subsets were measured. The potential to increase the T-helper cell concentration in blood is an important consideration when tapering corticosteroids. With hypothalamic–pituitary–adrenal axis suppression, these cells increase in number and may affect immune function and patient outcome.

In the multiple-dosing phase of our study, five of six subjects showed increases in cells above baseline at 24 to 32 hr. The sixth subject showed adrenal suppression but not the rebound effect. Rebound numbers increasing above that of baseline have been observed previously after corticosteroid therapy (16–18). Shoenfeld *et al.* (17) attributed it to an "increased egress . . . from the crowded bone marrow." Rebound may be explained by alterations in the number of affinity of endothelial adhesion molecules, which may occur due to prolonged glucocorticoid inhibition of adhesion molecule expression (2). Similar to our theory, Braat *et al.* (18) attributed rebound to cortisol suppression.

The pharmacodynamic modeling described by Braat *et al.* is limited because of their lack of cortisol and lymphocyte data between 12 and 24 hr. Additionally, their model assumes that drug distribution (i.e., an effect compartment) controls the response time lag which lacks consideration of the time course of efflux and return of cells to the blood.

The present dynamic modeling represents a two-part characterization of a type of tolerance phenomenon with the IC_{50} values of methylprednisolone and cortisol changing during continuous exposure to the former over 6 days. With more intensive study it might be possible to capture the time- or dose-dependence of these changes with an IC_{50} value expressed as a function such as $IC_{50}=f(C_{mp}, t)$. An analogous approach was taken to model the tolerance effects of cocaine by Chow *et al.* (19) using an exponential function for changing an EC_{50} value.

It is interesting to note that the ξ value, or methylprednisolone: cortisol potency ratio, is essentially the same for T-helper (15.9 ± 8.6) and T-suppressor (11.3 ± 8.4) cells. We have observed previously that apparent IC_{50} values for methylprednisolone show greater sensitivity ($IC_{50}=20$ ng/ml) for T-helper cells than for T-suppressor cells ($IC_{50}=112$ ng/ml). The previous modeling was done in normal subjects and did not account for the joint effects of methylprednisolone and cortisol. The differences compared to the present study results are probably related to the difference in operation of Eq. (2) when two agonists are present. Fractional Inhibition is determined by $C_{ant}/(IC_{50}+C_{ant})$. When only one agonist is used, the equation employs $C_{ant}=C_{mp}$ alone instead of $C_{ant}=C_{mp} \cdot \xi + C_{cort}$. With use of the latter, the same overall response requires a higher total IC_{50} to generate the same Fractional Inhibition as obtained with one more potent agonist. However, the IC_{50} values for T-helper and T-suppressor cells are similar in the present study which differs from our previous findings.

The present study employed total drug concentrations rather than unbound drug. This probably is not an important factor as methylprednisolone exhibits linear binding with a fraction unbound of about 0.24 (11). However, the lower cortisol concentrations produced after multiple dosing

of methylprednisolone could produce less unbound steroid because of the greater binding to transcortin occurring at low steroid concentrations. In turn, this might be factor contributing to the apparent tolerance of T-cell trafficking during multiple dosing. However, cortisol is much less contributory than methylprednisolone to the cell trafficking effects as judged from their relative IC_{50} values (see Table I).

This is the first model that concomitantly describes the circadian pattern of T-helper and T-suppressor cells as a function of endogenous cortisol concentrations, and the rebound in cell numbers after corticosteroid administration. The circadian rhythm of these cells was related to the circadian pattern of cortisol concentrations. The pharmacodynamic modeling also demonstrates that, with multiple dosing of corticosteroids, a decreased sensitivity of lymphocyte recirculation to corticosteroids, resulting in an increased IC_{50} value could account for rebound effects. Additionally, the increase or rebound in cells that occurs after only six daily doses of 20 mg methylprednisolone is partly explained by cortisol suppression, but the higher IC_{50} for cortisol makes this factor less contributory to the overall response.

ACKNOWLEDGMENTS

The advice of Dr. William F. Ebling and assistance of Ms. Nancy Pyszcynski, Mrs. Janice Pennington, and the Flow Cytometry Laboratory of Roswell Park Memorial Institute (Buffalo, NY) are greatly appreciated.

APPENDIX

Inhibition by Two Competitive Agonists (9)

Assume each of two drugs has an equal maximum effect, E_{max}, on a response variable

$$\frac{E}{E_{max}} = \frac{C}{EC_{50}(1 + A/EA_{50}) + C} + \frac{A}{EA_{50}(1 + C/EC_{50}) + A} \tag{A1}$$

where E is the effect, C and A are the concentrations of the agonists, and EC_{50} and EA_{50} are Michaelis-Menten constants for 50% inhibition. The differences in potency between C and A is expressed as ξ, a ratio equal to EC_{50}/EA_{50}. Substituting ξ in equation A1 yields

$$\frac{E}{E_{max}} = \frac{C}{EC_{50} + \xi \cdot A + C} + \frac{A}{EA_{50} + (C/\xi) + A} \tag{A2}$$

or

$$\frac{E}{E_{max}} = \frac{C}{EC_{50} + \xi \cdot A + C} + \frac{A \cdot \xi}{EC_{50} + C + A \cdot \xi} \tag{A3}$$

and simplifying

$$\frac{E}{E_{max}} = \frac{C + A \cdot \xi}{EC_{50} + (C + A \cdot \xi)} \tag{A4}$$

If the equation serves in an inhibitory model, the EC_{50} becomes IC_{50} and the fractional operation of the response variable is

$$I(t) = 1 - \frac{C + A \cdot \xi}{IC_{50} + C + A \cdot \xi} \tag{A5}$$

In the present application [Eq. (2)], C and A are concentrations of cortisol and methylprednisolone and IC_{50} is the concentration of cortisol which inhibits the return of lymphocytes to the blood by 50%. Equation (A5) can also be modified by the addition of a sigmoidicity power term.

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