Pharmacodynamics of Methylprednisolone Phosphate After Single Intravenous Administration to Healthy Volunteers

Hartmut Derendorf,[1,4] Helmut Möllmann,[2] Michael Krieg,[2] Sabine Tunn,[2] Carsten Möllmann,[1] Jürgen Barth,[2] and Hans-Jürgen Röthig[3]

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The pharmacokinetics and pharmacodynamics of methylprednisolone were investigated after intravenous administration of methylprednisolone phosphate to healthy subjects at seven different doses (16 to 1000 mg). Forty different pharmacodynamic parameters were followed for 1 week. The pharmacodynamic data were analyzed as a function of time as well as cumulative effects in form of the areas under the effect–time curves. Statistically significant dose-dependent effects of methylprednisolone were observed for 15 pharmacodynamic parameters. Highly significant ($P \leq 0.0001$) effects were increases in glucose levels, number of white blood cells, and segmented granulocytes as well as a decrease in the number of lymphocytes. For these pharmacodynamic effects an integrated pharmacokinetic/pharmacodynamic model was derived that translates the methylprednisolone plasma concentration–time profiles into effect–time profiles. This model allows prediction of pharmacodynamic effects for any single dose in the range studied at any time point.

KEY WORDS: methylprednisolone phosphate; pharmacokinetics; pharmacodynamics; lymphocytes; intravenous; granulocytes; glucose; healthy human subjects.

INTRODUCTION

In a recent study, we have investigated the pharmacokinetics and dose linearity of methylprednisolone and its prodrug methylprednisolone phosphate in a dose range from 16 to 1000 mg after intravenous administration to human volunteers (1). While there are numerous reports on effects of glucocorticoids such as changes in differential white blood-cell count and blood glucose levels (2–6), few studies correlate the pharmacodynamics with the steroid plasma levels. Boudinot *et al.* (7) devised a receptor-based model for prednisolone in the rat, with tyrosine aminotransferase activity as a pharmacodynamic end point. Oosterhuis *et al.* (8) investigated prednisolone-induced lymphocytopenia in humans after oral administration of doses between 10 and 60 mg and related the observed changes to prednisolone pharmacokinetics.

It was the purpose of the present study to screen a variety of blood parameters for a dose-dependent response after drug administration and describe the resulting effects as a function of time. Since the investigated compound is pharmacokinetically well characterized, it was further desired to develop an integrated pharmacokinetic-pharmacodynamic model that allows prediction of the expected pharmacodynamic changes as a function of time on the basis of the concentration–time profile in plasma.

METHODS

Clinical Studies. Methylprednisolone phosphate was administered intravenously in seven different doses between 16 and 1000 mg (1). There was a total of 12 healthy male subjects, randomly divided into two groups of 6. Group I received a dose of 31, 125, and 1000 mg. Group II received a dose of 16, 63, 250, and 500 mg. The subjects were 18–40 years old. Their body weight was 53–83 kg. Before the study they were examined. All laboratory values were in the normal range, and ECG and chest X-ray were normal. The study was carried out in conformance with the Declaration of Helsinki. All subjects were informed of the nature, purpose, and risk of the study and signed an informed consent form.

The injections of these high doses were tolerated reasonably well by the subjects. Immediately after the injection a burning and itching sensation was reported that lasted for up to 10 min.

All subjects were asked to abstain from strenous physical activity, beverages containing caffeine or other xanthine derivatives, nicotine, and alcohol from 36 hr before until 36 hr after drug administration. Drugs other than the study medication were not allowed from 1 week before beginning of the trial. On the day before the study subjects were asked to drink a volume of fluids of at least 1 liter between 6 and 10 PM in order to obtain a standardized basal situation. On the dosing day a standard breakfast was served 1.5 hr after drug administration. Blood sampling was performed on the contralateral arm with respect to drug administration. Blood samples for pharmacokinetic analysis were drawn immediately before dosing and at appropriate points up to 48 hr after dosing. Methylprednisolone and its phosphate ester were analyzed in plasma by HPLC (1,9). Blood samples for pharmacodynamic measurements were drawn immediately before dosing and at 0.2, 4, 12, 24, 48, and 168 hr after dosing. Forty different parameters were measured (Table I).

Pharmacokinetic Analysis. The results of the pharmacokinetic studies have been reported elsewhere in detail (1). For the correlation of pharmacokinetics and pharmacodynamics, the data were converted to calculate the free methylprednisolone concentration in tissues as an estimate of the actual active concentration at the receptor site (10,11). Plasma concentration–time curves of methylprednisolone were fitted to a three-exponential equation:

$$C_p = A * e^{-\alpha t} + B * e^{-\beta t} + C * e^{-k_a t} \qquad (1)$$

with the constant coefficients $A + B + C = 0$, the first-order constant k_a to quantify the rate of phosphate conversion, and the two hybrid constants α and β to quantify drug

[1] Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, Florida 32610.
[2] Medical Clinic "Bergmannsheil," University of Bochum, 4630 Bochum, West Germany.
[3] Hoechst AG, 6230 Frankfurt, West Germany.
[4] To whom correspondence should be addressed at Box J-494, Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, Florida 32610.

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Table I. Investigated Pharmacodynamic Parameters that Showed a Statistically Significant Effect of the Dose as Indicated by ANOVA ($P \leq 0.05$), Baseline Values, and Maximum Effects (Means ± SD) Observed After Administration of the Highest Dose Investigated (1000 mg)

Parameter	Baseline	Maximum effect
Mean corpuscular hemoglobin (fmol)	1.95 ± 0.09	1.91 ± 0.09
Hematocrit	0.450 ± 0.036	0.416 ± 0.022
White blood-cell count (G/L)	6.45 ± 1.85	17.45 ± 4.26
Eosinophils (%)	2.2 ± 1.3	0.0
Eosinophils (G/L)	0.145 ± 0.116	0.000
Bands (stab cells (%)	0.3 ± 0.5	2.5 ± 2.7
Bands (stab cells) (G/L)	0.015 ± 0.026	0.436 ± 0.510
Segmented cells (%)	48.4 ± 5.7	86.5 ± 4.8
Segmented cells (G/L)	3.08 ± 0.92	13.78 ± 3.63
Lymphocytes (%)	40.5 ± 4.1	10.6 ± 4.5
Lymphocytes (G/L)	2.63 ± 0.80	1.09 ± 0.49
Monocytes (%)	8.2 ± 2.6	0.8 ± 1.0
Monocytes (G/L)	0.538 ± 0.238	0.087 ± 0.098
Fasting blood sugar (mmol/L)	5.01 ± 0.81	8.34 ± 1.45
Triglycerides (mmol/L)	1.47 ± 1.21	0.56 ± 0.15
Sodium (mmol/L)	142.1 ± 2.1	140.3 ± 2.4
Uric acid (μmol/L)	341.1 ± 36.7	274.4 ± 53.4
Urea (mmol/L)	5.02 ± 1.35	6.88 ± 1.65
Cortisol (μg/dl)	14.79 ± 5.95	0.81 ± 1.47
Beta-globulin (%)	12.74 ± 1.93	11.59 ± 1.71

distribution and elimination. Maximum tissue concentrations are reached at t_{max}:

$$t_{max} = \ln [\alpha(k_a - \beta)/\beta(k_a - \alpha)]/(\alpha - \beta) \quad (2)$$

The total amount of drug in the tissues at that time point $X_T(t_{max})$ can be calculated by substitution of t_{max} into Eq. (3) (11):

$$X_T = Z * [(\alpha - \beta)e^{-k_a t} + (\beta - k_a)e^{-\alpha t} + (k_a - \alpha)e^{-\beta t}] \quad (3)$$

with

$$Z = k_a * f * D * k_{12}/[(\alpha - \beta) * (\alpha - k_a) * (\beta - k_a)]$$

where D is the dose, f is the fraction of phosphate converted to methylprednisolone, and k_{12} is the microconstant describing the rate of transfer from the central to the peripheral compartment which can be calculated from Eq. (1) (12).

Since at t_{max} the free concentrations in plasma and tissues are equal, combination of Eqs. (1)–(3) and the fraction bound to plasma protein (f_b) allows calculation of the free concentration in the tissues (C_T^f):

$$C_T^f = X_T * C_p(t_{max}) * (1 - f_b)/X_T(t_{max}) \quad (4)$$

Statistical Analysis. Statistical analysis was performed using the program SAS/STAT (13). Differences were considered to be significant for $P \leq 0.05$. Statistical evaluation was done for the individual pharmacodynamic data using the SAS General Linear Models (GLM) procedure for repeated-measures analysis of variance as well as for the respective area under the effect/time curves (AUCs) using the GLM analysis of variance. In case of a significant effect of the dose on AUC the ANOVA was followed by a Scheffe multiple-comparison test.

Integrated Pharmacokinetic-Pharmacodynamic Model. The free tissue concentration–time profiles obtained in the pharmacokinetic analysis were used as estimates for free methylprednisolone concentrations at the receptor site as a function of time. A simple E_{max} model (14,15) was used to relate these concentrations (C_T^f) to the respective effects (E):

$$E = E_0 + E_{max} * C_T^f/(E_{50} + C_T^f) \quad (5)$$

where E_0 is the baseline, placebo level of the pharmacodynamic indicator, E_{max} the maximum effect that can be achieved, and E_{50} the concentration that will produce half of the maximum effect. Since there is a significant delay in the onset of the effects, a lag time (t_{lag}) was added to the model to shift the effect–time curve.

Several, more complex, pharmacodynamic models including effect compartments were investigated to convert these concentrations into effect–time profiles. However, none of these models provided significantly better fits to the experimental data than the simple E_{max} model.

The data were fitted by nonlinear regression using the program MINSQ (16). For each pharmacodynamic parameter tested, all data for all doses were fitted simultaneously in a single pharmacokinetic/pharmacodynamic model.

Cumulative Pharmacodynamic Effects. Areas under the effect–time curves (AUC) were utilized to correlate the cumulative pharmacodynamic effects with dose. AUCs were calculated by the trapezoidal rule as the area above baseline (E_0) between 0 and 48 hr.

RESULTS

Pharmacokinetic Analysis. The pharmacokinetic results of this study have been reported in detail (1). Over the 60-fold dose range investigated, no nonlinearity in the total-body clearance of methylprednisolone phosphate or methylprednisolone could be detected. The average elimination half-line for the prodrug was 3.7 min, indicating rapid hydrolysis. Very little of the ester (average, 0.9% of the dose) was excreted unchanged into the urine and hence not bioavailable. Methylprednisolone is formed rapidly. The total-body clearance was 21 liters/hr, and the terminal half-life 2.8 hr. The plasma protein binding is 78%, with no apparent nonlinearity in the investigated dose range.

For the purpose of the present study the free concentrations in the tissues were calculated (Fig. 1). Maximum tissue levels are obtained after 31 min (t_{max}), indicating a rapid distribution of the generated methylprednisolone. The peak free concentration in tissue is equal to the free plasma level at t_{max} and is proportional to the dose with a proportionality factor of 1.85 (dose as mg, concentrations as ng/ml). Hence, for the highest dose of 1000 mg methylprednisolone phosphate, the peak free tissue level of methylprednisolone was 1.85 μg/ml. Figure 1 shows the complete concentration–time profile in plasma and tissue for the 1000-mg dose.

Pharmacodynamic Analysis. Fifteen of the 40 pharma-

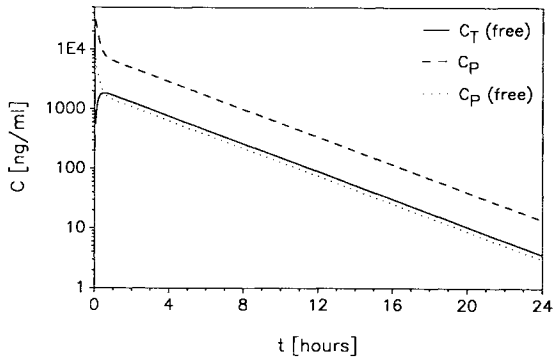

Fig. 1. Plasma concentration, C_p, free plasma concentration, C_p (free), and free tissue concentration, C_T (free), of methylprednisolone as a function of time after intravenous administration of 1000 mg methylprednisolone phosphate.

codynamic parameters tested (Table I) showed a statistically dose-dependent effect. The most profound effects observed were increases in blood glucose, total white blood cells, and specifically segmented granulocytes as well as decreases in lymphocyte count and endogenous hydrocortisone. Other sensitive parameters include leukocyte subclasses (eosinophils, monocytes, bands), sodium, uric acid, urea, triglycerides, hematocrit, MCH, and β-globulin. The effect on endogenous hydrocortisone has already been discussed elsewhere (1).

Integrated Pharmacokinetic Pharmacodynamic Model. The proposed model allowed excellent predictions, with correlation co-efficients above 0.9 for the parameters glucose levels, lymphocyte count, and number of segmented cells. Reasonable predictions (correlation coefficients, 0.6–0.9) were further possible for the parameters white blood-cell count, triglycerides, sodium, and uric acid. The estimated pharmacodynamic parameters are listed in Table II. It should be emphasized that the presented model integrates information for all doses at all time points.

Fasting Blood Sugar. Glucose levels increased as a function of dose. The effect is highly significant at 4, 12, and 24 hr. Figure 2 shows the model-predicted glucose levels for each dose as well as the experimental data. The average baseline glucose level was 4.87 mmol/liter, and the average maximum effect was an increase to 9.44 mmol/liter, hence almost a twofold increase in glucose concentrations.

Analysis of the areas under the curve also shows highly significant dose dependency. The multiple-comparison test showed significant differences for the AUCs between 1000 mg and doses up to 125 mg. The 500- and 250-mg doses are significantly different from doses up to 64 mg.

Lymphocytes. The number of lymphocytes, expressed as a fraction of total white blood cells, is clearly decreased as a function of dose. The integrated pharmacokinetic/pharmacodynamic model allowed an excellent prediction of lymphocyte count (Fig. 3). The average baseline lymphocyte count was 0.390; the average maximum effect was a decrease to 0.112. However, since these numbers are fractions of total white blood cells, they need to be converted to calculate the total number of lymphocytes. The baseline count averaged 2.46 G/liter and was decreased to about 0.97 G/liter, hence a decrease to less than half their baseline number. Since the concomitant increase in total white blood-cell count amplified the percentage decrease in lymphocyte count, the relative values showed a better fit to the model than the absolute cell count.

Analysis of the areas under the curve showed highly significant dose dependency. The multiple-comparison test indicated significant differences of the AUCs (relative counts) for 500 and 1000 mg with doses up to 64 mg; doses of 64 mg and more are significantly different from placebo. For the absolute counts 1000 mg was significantly different from doses up to 250 mg.

Segmented Cells. Segmented granulocytes showed a drastic increase in number as a function of dose. The effect is significant 4, 12, and 24 hr after drug administration. The model allowed an excellent prediction of cell count for all doses studied (Fig. 4). The average baseline level (fraction of total WBC count) was 0.519; the average maximum effect was an increase to 0.844. As in the case of the lymphocytes these numbers need to be converted to absolute cell counts. The total baseline count averaged 3.2 G/liter and was increased to 8.91 G/liter, hence an increase of almost threefold. A better fit to the model was obtained with the relative cell count (correlation coefficient, 0.959 vs 0.881), but in either case a good prediction of the effect–time profile was possible.

Analysis of the areas under the curve for the relative count showed significant differences of the AUCs for 500 and 1000 mg with doses up to 32 mg; doses of 125 mg and more were significantly different from placebo. For the absolute count the 1000-mg dose was significantly different for doses up to 125 mg; doses of 64 mg and more are significantly different from placebo.

Other Pharmacodynamic Parameters. The changes of the other significantly dose-dependent parameters (Table I)

Table II. Pharmacodynamic Constants for the Integrated Model[a]

	WBC	SEGM	SEGM[b]	LYMP	LYMP[b]	FBS	TRIG	SODI	UAC
E_{max}	5.49	0.325	5.71	−0.278	−1.49	4.57	−0.61	−1.90	−61.4
E_{50}	0.13	1.84	0.76	1.32	9.7	22.6	7.68	1.94	0.42
E_0	6.08	0.519	3.20	0.390	2.46	4.87	1.19	141.9	306.8
t_{lag}	3.99	3.96	3.99	3.97	3.24	3.99	3.99	3.73	11.6
Corr.	0.831	0.959	0.881	0.959	0.798	0.938	0.684	0.658	0.698

[a] WBC, white blood cells; SEGM, segmented cells; LYMP, lymphocytes; FBS, fasting blood sugar; TRIG, triglycerides; SODI, sodium; UAC, uric acid; Corr., correlation coefficient. All units for E_{max} and E_0 as in Table I; E_{50} as ng/ml; t_{lag} as hr.
[b] Absolute cell counts.

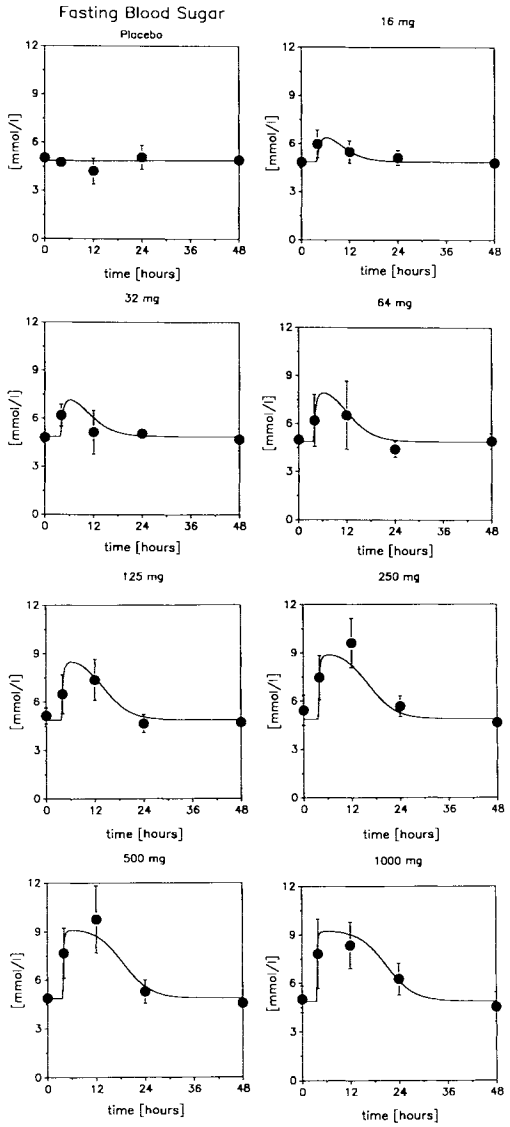

Fig. 2. Increase in glucose levels for the different doses studied. The curves represent the model predicted changes in glucose levels; the points (±SD) are the experimentally measured values.

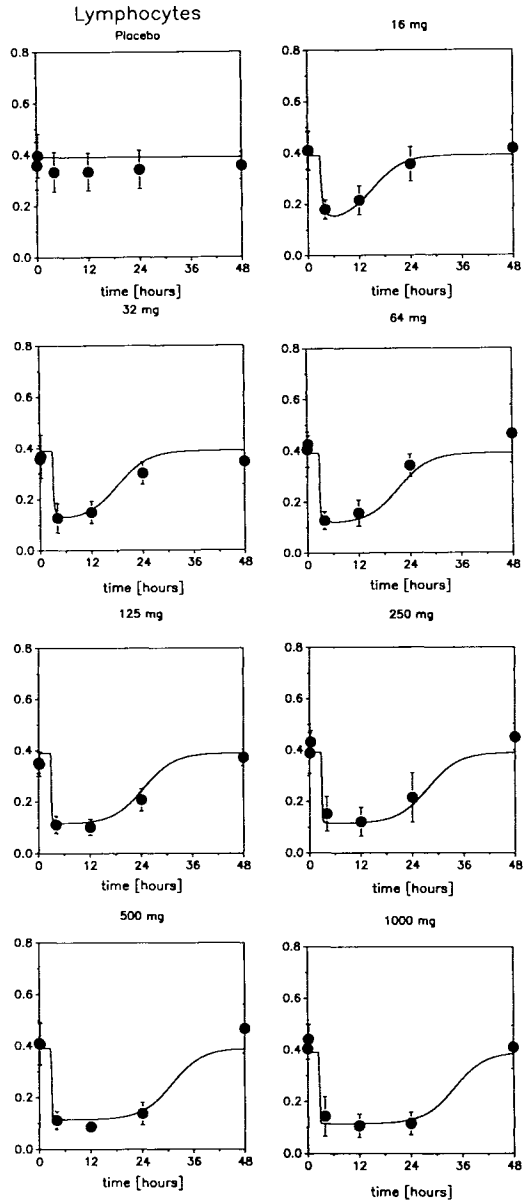

Fig. 3. Decrease in lymphocyte count for the different doses studied. The curves represent the model predicted changes in relative lymphocyte count; the points (±SD) are the experimentally measured values.

could not be fitted to the model with the same accuracy; the correlation coefficients were below 0.9.

Cumulative Effect-vs-Dose Relationship. As indicated by Figs. 2–4, there is no linear relationship between dose and maximum effect for the presented pharmacodynamic parameters. Maximum possible effects (E_{max}) are already achieved with relatively small doses and an increase in dose leads mainly to a prolonged duration of the effect. However, there is a linear relationship between the logarithm of the dose and the cumulative effects quantified by the area under the effect–time curve (Fig. 5). The respective slopes, intercepts, and correlation coefficients are 41.9, −46.2, and 0.791 for glucose levels, −3.66, 0.605, and 0.919 for lymphocyte count, and 4.13, 0.58, and 0.911 for segmented cells. This simple relationship is valid for doses where the maximum effect is achieved. If these doses are doubled, the duration of the maximum effect will be extended for one half-life (ln $2/\beta$), and hence, the AUC will be enlarged by ln $2 * E_{max}/\beta$. The relationship allows quick predictions for cumulative effects with respect to dosage changes.

DISCUSSION

The presented pharmacokinetic/pharmacodynamic model allows the interpretation of the available pharmacokinetic information for methylprednisolone (1), since the concentration–time profiles can now be converted into effect–time profiles. It could be shown that, for most of the sensitive parameters, even with the lowest of the investigated doses maximum effects were achieved and that dose increases resulted in a prolongation of the effect but not in a further increase in intensity. This could be explained simply

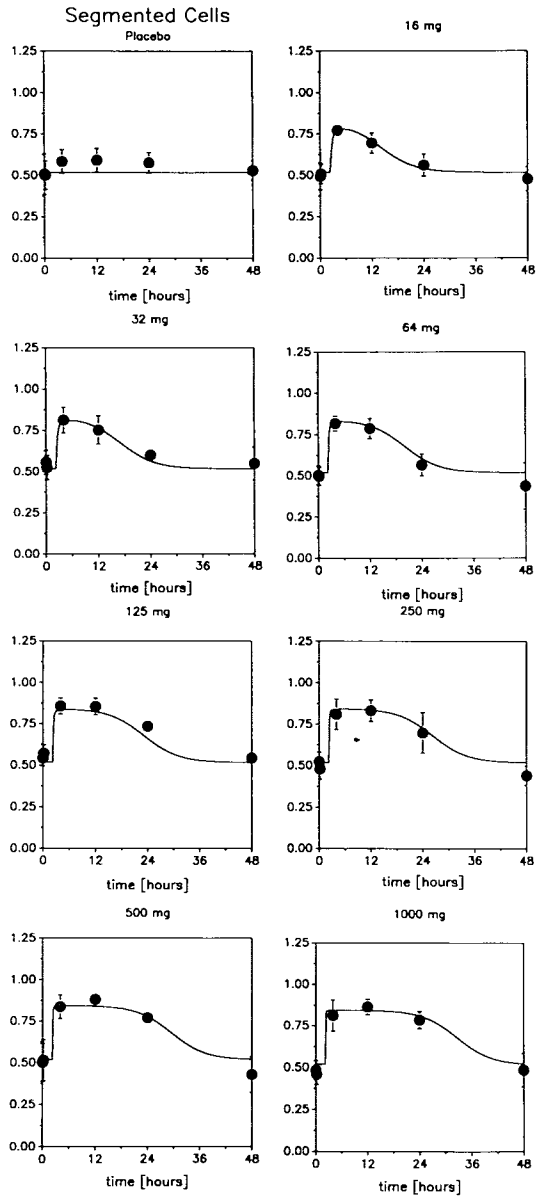

Fig. 4. Increase in the number of segmented cells for the different doses studied. The curves represent the model predicted relative changes in the number of segmented cells; the points (±SD) are the experimentally measured values.

by the pharmacokinetic properties of methylprednisolone; drug elimination was rate limiting for the disappearance of the effect. Hence, the intensity of the response is limited by the steroid receptor binding capacity, while the duration of the response is determined by drug elimination.

Further, the determination of free tissue levels allowed the estimation of the active drug concentration at the receptor site. The affinity of the glucocorticoid receptor for steroid is high: at concentrations of about 10^{-8} M, 50% saturation of receptors occurs (17,18). This is equivalent to a methylprednisolone concentration of about 4 ng/ml. Although there is considerable variability, this number is in good agreement with the E_{50} values reported in Table II, which are the free concentrations in tissues for 50% of the

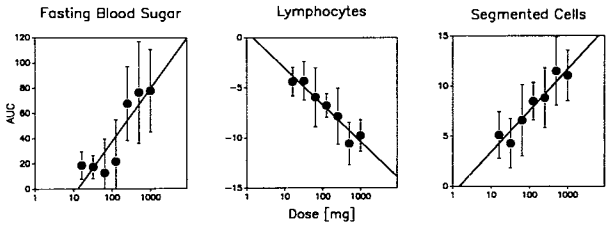

Fig. 5. Linear relationship between the logarithm of the dose and the cumulative effect as measured by the area under the effect–time curve for increase in glucose, decrease in lymphocyte count, and increase in number of segmented cells.

maximum effect. Hence, the proposed model is able to link the pharmacokinetics to the pharmacodynamics using experimental receptor-binding data rather than using a hypothetical effect compartment.

It is remarkable that the derived pharmacokinetic-pharmacodynamic model was operative over a 60-fold dose range and that the same model could be used for different pharmacodynamic parameters. The latter result supports the theory that the mechanism of action for the glucocorticoid effects on the investigated parameters is mediated via the same receptor. Deviations from the model were observed for the very high doses of methylprednisolone for the effect on total white blood-cell count. The model consistently underestimated the experimentally measured values at 24 hr, indicating that maybe other additional mechanisms of action are involved.

More work is needed to characterize the onset of activity accurately. In the present study not enough data points were collected during the first 4 hr. It will be interesting to see if the model can similarly be applied to other glucocorticoids. This would allow a comparison of different glucocorticoids where the pharmacokinetic and pharmacodynamic contributions to the overall effects could be separately evaluated. Furthermore, it is not clear if the presented relationships will also be applicable after multiple dosing of glucocorticoids. Answers to these questions should lead to a more rational dosage design for glucocorticoids under consideration of the respective steroid-specific pharmacokinetic and pharmacodynamic properties.

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