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Concentration-Dependent Effects of Prednisolone on Lymphocyte Subsets and Mixed Lymphocyte Culture in Humans

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ABSTRACT

In a previous study the feasibility of pharmacokinetic-pharmacodynamic modeling for a quantitative description of the lymphocytopenic effect of prednisolone was demonstrated. We now applied this technique to compare the lymphocytopenia of T-lymphocyte subsets, namely CD8 and CD4. The finding of similar rate constants for the delay of the effect on different T-lymphocyte categories supports the explanation of this delay on the basis of pharmacokinetics rather than cellkinetics. The time course of the responsiveness of remaining lymphocytes in mixed lymphocyte culture after prednisolone administration could be described with the same model as the lymphocytopenia. The concurrence of both effects suggests that total lymphocyte counts, if considered intraindividually, could be used as a measure for monitoring the indirect immunosuppressive effect of prednisolone. The inhibitory effect on mixed lymphocyte culture of plasma from subjects who received prednisolone was directly related with the prednisolone concentrations in plasma. Just as for the indirect effect, a threshold concentration could be observed in the concerning concentration-effect relation. This is attributed to the decrease of endogenous hydrocortisone levels under the influence of prednisolone. Possible consequences of our results and those from related studies for the use of prednisolone as an immunosuppressive drug are discussed.

The optimal clinical use of prednisolone as an immunosuppressive agent has thus far been hindered by the lack of a rational basis for the choice of dosage regimens. In several clinical studies, regimens using "high" versus "low" prednisolone doses have been compared for their therapeutic efficacy, and no significant differences could be demonstrated (McGeown *et al.*, 1980; Nelson *et al.*, 1982; Papadakis *et al.*, 1983; Park *et al.*, 1984). One limitation in this respect has been overcome in recent years by the improved insight in the complicated pharmacokinetic profile of prednisolone (Rose *et al.*, 1981).

However, quantification of the immunosuppressive effect is still difficult, and its relationship to prednisolone concentrations is poorly understood. Extensive study has shown several effects of prednisolone and other corticosteroids on certain components of the immune response in humans (Cupps and Fauci, 1982). An important role is ascribed to T-lymphocytes, which regulate both humoral and cellular immunity, and act as effector cells in cellular immunity.

It seems useful to quantify the effects on these lymphocytes in order to relate the pharmacokinetic profile of prednisolone with the time course of its pharmacodynamics, *i.e.*, its immunosuppressive action. Indeed, Cochrum *et al.* (1974) demonstrated that the reactivity of MLCs is of predictive value with respect to the survival of human renal allografts. Frey *et al.* (1980, 1982) studied the pharmacodynamics of the inhibition of the MLC by plasma of patients or healthy subjects in relationship to the pharmacokinetics of prednisolone (Frey *et al.*, 1980, 1982; Frey and Frey, 1984). In a recent study they also took into account some immunological parameters that are related to the redistribution of lymphocytes. Due to a time delay, they found no satisfactory quantitative relationship between prednisolone concentrations and these parameters (Frey *et al.*, 1984).

In recent years a new approach had been introduced to the quantitative description of the relation between the time course of the serum concentration of a drug and the time course of reversible effects by integrated pharmacokinetic-pharmacodynamic modeling (Holford and Sheiner, 1982). In a previous study we applied this approach to the effects of prednisolone on the CD3 and CD4 lymphocyte counts in peripheral blood (Oosterhuis *et al.*, 1984). In the present work we applied this model to describe and compare the effects of prednisolone on the overall lymphocytopenia and the redistribution of CD8

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ABBREVIATIONS: MLC, mixed lymphocyte culture; V_d, apparent volume of distribution; F, bioavailability; AUC, area under the serum concentration-time curve; Cl, body clearance; AUEC, area under the effect-time curve; Cth, threshold concentration.

lymphocytes. The relation between the time course of lymphocytopenic effects and the proliferative responses of remaining lymphocytes in MLC was also investigated. Finally, the influence of plasma, obtained at different time intervals after prednisolone administration, on the proliferative response of unredistributed lymphocytes was taken into account. The data presented in this study were collected at the same time as the previously reported data. Possible clinical consequences of our results and the results from related studies are discussed.

Methods

Subjects and sample collection. The protocol of the study was reviewed by the ethical committee of our hospital. After informed consent was obtained, 15 healthy volunteers, age 27 to 45 years, received a single p.o. dose of prednisolone at 8:00 A.M. They were randomly divided into three groups of five subjects, who received doses of 10, 30 and 60 mg, respectively. Prednisolone was administered as 5-mg tablets, manufactured by the hospital pharmacy. None of the subjects received any other drug during the period of this study. Venous blood was drawn before intake of the drug, at hourly intervals for the first 6 h, and next at 8, 10, 11, 12 and 24 h after drug administration. The sera obtained from each individual were frozen at −20°C for determination of corticosteroid levels.

Corticosteroid analysis. Prednisolone, prednisone and hydrocortisone were analyzed simultaneously by a high-performance liquid-chromatography assay as published by Rose and Jusko (1979), with modifications as described previously (Oosterhuis *et al.*, 1984). The coefficients of variation were approximately 5 and 8% for prednisolone and hydrocortisone, respectively. The lower limit of detection for each compound was about 10 ng/ml in serum.

Lymphocyte counts in peripheral blood. The methodology used for blood cell counting and differentiation has been described in detail previously (ten Berge *et al.*, 1984; Van de Griend *et al.*, 1981). Absolute lymphocyte counts were calculated from total leukocyte counts after differentiation on blood smears. Mononuclear cells were isolated with density gradient centrifugation using Ficoll-Hypaque (resulting in a fraction that contains 90% mononuclear cells of which 80% are lymphocytes and 20% are monocytes). T lymphocyte subsets were analyzed with an indirect immunofluorescence technique, using monoclonal antibodies of the OKT series (Ortho Pharmaceutical Laboratories, Beerse, Belgium), *i.e.*, OKT4 (CD4) and OKT8 (CD8) (ten Berge *et al.*, 1984). Quantification was by flow cytometry after gating out the monocytes (Epics-C, Coulter Electronics, England). The absolute counts for the subsets were calculated by multiplying the percentage of antibody-binding cells with the absolute lymphocyte counts. The lymphocytopenic effects were expressed as the percentage of decrease of absolute counts in peripheral blood with respect to the counts at $t = 0$. These values will be referred to as the total lymphocytopenic, the CD4 and the CD8 lymphocytopenic effect, respectively.

Lymphocyte cultures. MLCs were mainly performed as described previously (ten Berge *et al.*, 1984). The influence of lymphocyte redistribution on the proliferative response of remaining lymphocytes was investigated with the following set-up. Standard numbers of lymphocytes (40,000, as obtained with density gradient centrifugation at the different time points after prednisolone administration), were cultured in Roswell-Park Memorial Institute-1640 medium supplemented with 20% pooled human serum. Human lymphocytes (40,000), from different donors and irradiated with 2000 rad, were used as stimulant. The percentage of decrease of the proliferative response with respect to the cultures of cells, obtained before prednisolone administration, is designated as the cellular MLC effect. To avoid day-to-day variations in the proliferative assays, all cultures were performed in one experiment using the same sample of irradiated lymphocytes for stimulation.

Mean data curves were calculated for the different effects by averaging the observations for individuals within a dosage group, at each time point. The direct effect of prednisolone in plasma on the MLC response of lymphocytes was measured with a similar MLC set-up as described above. However, in this case standard numbers of unredistributed lymphocytes obtained at $t = 0$ were used and the culture medium was supplemented with 20% plasma from the individual, obtained at the different time points after prednisolone administration. Again, the effect was expressed as percentage of decrease of the proliferative response with respect to the cultures containing plasma from $t = 0$. This effect is designated as the plasma MLC effect.

Data analysis. The pharmacokinetic and pharmacodynamic parameters were estimated by the nonlinear least-square computer program NONLIN (Metzler *et al.*, 1974). Unit weighting was used for all observed values. The serum concentration curves of prednisolone were fitted to a biexponential equation, representing a one-compartment model with first order absorption. Thus, the absorption rate constant (k_a), the elimination rate constant (K) and the ratio (V_d/F) of the V_d and the F were estimated. From the estimated pharmacokinetic parameters, the AUC and the ratio of Cl and F were derived according to standard pharmacokinetic concepts (Gibaldi and Perrier, 1975). The equation, based on the work of Muldoon and Westphal (1967) with mean protein-binding parameters from 12 subjects as published by Frey *et al.* (1982) was used to estimate the protein-free concentrations corresponding with the mean prednisolone serum concentration data of the three dosage groups. The area under the hydrocortisone concentration *vs.* time plot from 1 to 12 h after administration of prednisolone was calculated with the trapezoidal rule (Gibaldi and Perrier, 1975). The following equation was used to describe the time course of the concentration in the hypothetical effect compartment, as derived by Holford and Sheiner (1982):

$$Ce = \frac{\text{dose} \cdot k_a \cdot k_{e0}}{V_d}\left[\frac{e^{-K \cdot t}}{(k_a - K)\,(k_{e0} - K)} + \frac{e^{-k_a \cdot t}}{(K - k_a)\,(k_{e0} - k_a)} + \frac{e^{-k_{e0} \cdot t}}{(K - k_{e0})\,(k_a - k_{e0})}\right] \quad (1)$$

In this equation, t is hours after drug administration; k_{e0} is the rate constant of prednisolone elimination from the hypothetical effect compartment. In fact, Ce is the serum concentration at steady state which is at any time proportional with a certain effect compartment concentration. For the pharmacodynamic model, relating Ce with effect, we used a modification of the basic E_{max} model represented by the following equation:

$$E = \frac{E_{max}\,(Ce - Cth)}{(EC_{50} - Cth) + (Ce - Cth)} \quad (2)$$

By definition, the effect is zero when Ce is less than a certain threshold concentration Cth. A steep onset of effect is predicted when Ce becomes greater than Cth. The EC_{50} parameter represents the Ce that corresponds with 50% of the maximal effect (E_{max}). The lymphocytopenic and the cellular MLC effect data were fitted on this threshold E_{max} equation, in which the right-hand side of equation 1 was substituted for Ce, using the previously estimated pharmacokinetic parameter as fixed values. Thus, the parameters K_{e0}, EC_{50} and Cth were estimated with E_{max} being fixed at 100%. The latter was justified by the exploration and validation of the model (Oosterhuis *et al.*, 1984). The AUECs was calculated by numerical integration of the fitted curves, using the trapezoidal rule ($\Delta t = 0.5$ h). The pharmacodynamic parameters of the CD 4 and the CD8 lymphocytopenic effect were compared by the paired t test. Coefficients of correlation for linear relationships were calculated by linear regression analysis.

Results

Corticosteroid levels. The time courses of prednisone and prednisolone plasma concentrations were similar, the ratio prednisone/prednisolone being about constant at any time within one individual. The time course of prednisolone concentrations in the individual subjects as well as the mean data for

each dosage group could adequately be fitted to the pharmacokinetic model. With an increase of the dose from 10 to 30 mg a significant increase of V_d/F and of Cl/F was observed ($P < .01$). From 30 to 60 mg this was true for V_d/F ($P < .05$). Only after correction of the mean data for the protein bound fraction, a significant linear correlation with a mall intercept was found between dose and AUC (AUC = 14.5 × Dose + 14.1; $r = 0.9993$, $P < .05$). Total hydrocortisone levels in serum decreased to approximately 5 to 15% of their initial values within 1 to 2 h after the administration of prednisolone, and subsequently remained at a rather constant level during at least 10 h.

Lymphocytopenic effects. A pronounced counterclockwise hysteresis was observed, when the mean lymphocytopenic effects were plotted against the corresponding mean serum concentrations (not shown), which may indicate a delay of the concentration profile at the receptor site with respect to the serum concentration profile of prednisolone. The observed time course of the lymphocytopenic effects could be fitted to the proposed pharmacokinetic-pharmacodynamic model. This is illustrated for the 30-mg dosage group in figure 1. The influence of the dose on the time course of these effects is illustrated for the total lymphocytopenic effect in figure 2. The total lymphocytopenic and CD8 lymphocytopenic effect curves of the individual subjects were fitted analogously, and the estimated pharmacodynamic parameters and k_{e0} are presented in table 1.

The parameters of the CD8 lymphocytopenic effect, considered over the three dosage groups, in table 1 were tested for differences with respect to the previously published parameters of the CD4 lymphocytopenic effect (Oosterhuis *et al.*, 1984). The average EC_{50} (±S.D.) for the CD8 lymphocytopenic effect (165.3 ± 62.4) was significantly higher than for the CD4 lymphocytopenic effect (132.0 ± 39.2; $P < .01$, $n = 14$; paired t test), the mean difference being 33.3 ng/ml. This corresponds to a significantly smaller AUEC (485 ± 136) for the CD8 lymphocytopenic effect when compared with the CD4 lymphocytopenic effect (597 ± 175; $n = 14$, $P < .02$; paired t test). The average (±S.D.) Cth for the CD8 and CD4 lymphocytopenic effects were 95.7 ± 50.8 and 93.9 ± 36.4, respectively. The average k_{e0} was 0.292 ± 0.091 for the CD8 lymphocytopenic effect and 0.293 ± 0.076 for the CD4 lymphocytopenic effect. The latter two parameters for the CD8 and CD4 subpopulations were statistically not different and in fact very similar. There were no significant differences between the parameters of the total lymphocytopenic and the CD8 lymphocytopenic effect in table 1 (paired t test). To illustrate that consistent results are obtained with the model, the pharmacodynamic parameters as found by fitting the mean data curves of the lymphocytopenic and CD8 lymphocytopenic effect are given in table 2. The parameters based on total prednisolone serum concentrations are in good agreement with the mean parameters from the individual fits in table 1.

Proliferative responses. The time course of the cellular MLC effect (inhibition of the proliferative response of peripheral lymphocytes obtained at the different time points after prednisolone-administration) was roughly concurrent with lymphocytopenic effect curves. When the mean data on this cellular MLC effect of the dosage groups from the different time points (Y) were plotted against the corresponding CD4 lymphocytopenic effect data (X), a significant linear correlation was found and a small intercept ($Y = 0.7094X - 5.225$, $r = 0.8598$, $n = 30$; $P < .001$). The correlation plot is shown in figure 3. Similar correlations were found between the cellular MLC effect and the total lymphocytopenic effect ($r = 0.8405$) or the CD8 lymphocytopenic effect ($r = 0.8028$). Due to the variation in the MLC effect measurements, only mean data of the dosage groups were fitted to the pharmacokinetic-pharmacodynamic model. As an example, the fit of the mean cellular MLC effect curve for the 30-mg dosage group is shown in figure 4. The resulting pharmacodynamic parameters of the cellular MLC effect for the dosage groups are also presented in table 2. In comparison with the lymphocytopenic effects, Cth and k_{e0} are again very similar. The response in the MLC of lymphocytes obtained before prednisolone administration was depressed when 20% plasma of the different time points after prednisolone administration was present in the culture medium (plasma MLC effect). The time course of this plasma MLC effect was roughly parallel to the prednisolone concentration profile. In figure 5, the mean data of this plasma MLC effect for the 60-mg dosage group are plotted against the corresponding mean serum concentrations (total prednisolone). The data were fitted

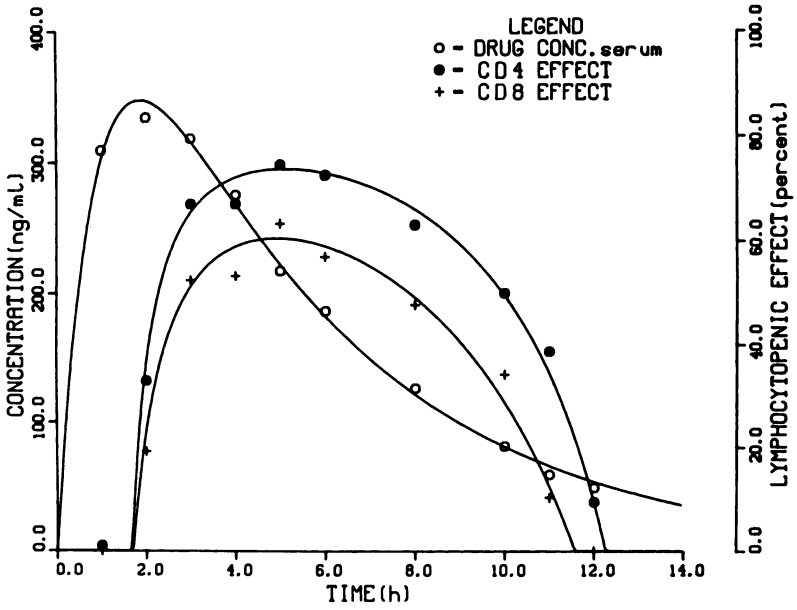

Fig. 1. Time course of the prednisolone serum concentration (CONC) and the CD4 and CD8 lymphocytopenic effects; mean data of the 30-mg dosage group. Drawn lines represent fits to pharmacokinetic and integrated model.

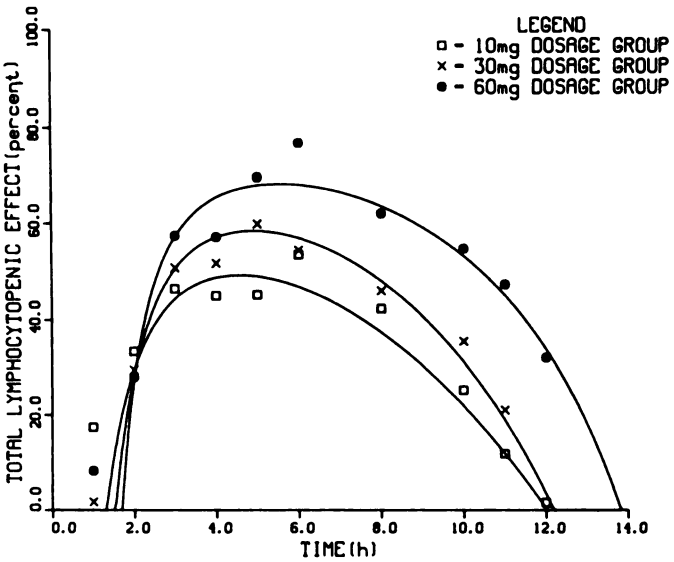

Fig. 2. Mean data curves of the total lymphocytopenic effect for the three dosage groups. Drawn lines represent fits to integrated model.

to the threshold E_{max} equation (equation 2) in which the total prednisolone serum concentration was substituted for *Ce*. The threshold concentration and EC_{50} parameters were estimated at 134.4 and 840.2 ng/ml, respectively. A similar fit using the calculated free prednisolone concentrations in the cultures yielded a threshold of 7.3 and an EC_{50} of 104.7 ng/ml, corresponding with total serum concentrations of 98.0 and 785.0 ng/ml, respectively.

Discussion

The pharmacokinetic results are in agreement with the opinion that prednisolone shows dose-dependent kinetics due to nonlinear plasma-protein binding (Rose *et al.*, 1981). Nevertheless, the time course of prednisolone serum concentrations could adequately be fitted to the linear pharmacokinetic model. The decrease of endogenous hydrocortisone levels after prednisolone administration has been reported frequently (Rose and Jusko, 1979; Frey *et al.*, 1981). This is of importance for the evaluation of the effects of prednisolone, as it is known that hydrocortisone exerts similar effects on lymphocyte redistribution and proliferative responses (Cupps and Fauci, 1982; Fauci and Dale, 1974).

The time course of the effects in the figures 1 and 2 illustrate how the lymphocytopenic effect was extinguished, whereas substantial prednisolone serum concentrations were still measured. In relation with these observations we have argued previously that the threshold parameter in the pharmacodynamic component of our model represents a prednisolone concentration (in the effect compartment) that substitutes for a depleted hydrocortisone concentration (Oosterhuis *et al.* 1984, 1986). A basic assumption to the model is that the effects on lymphocyte redistribution by prednisolone and hydrocortisone are concentration dependent and reversible. As a consequence the delay between measured concentrations and effect is to be attributed to the fact that the concentration profile at the effect site lags behind the serum concentration profile of prednisolone. Alternatively, one could try to explain the delay on the basis of cellkinetics (time required for redistribution of cells within the body) or a combination of both factors. The finding of a similar k_{e0} for the different lymphocyte categories makes a cellkinetic explanation less probable, as it is unlikely that the different subpopulations would behave identical in this respect. Additional experiments are in progress in our laboratory which seem to indicate that distribution of labeled lymphocytes within the body occurs very fast, so that cellkinetics are not a rate limiting factor in these effects of prednisolone.

Figure 1 demonstrates that the CD4 and the CD8 lympho-

TABLE 1

Pharmacodynamic parameters and K_{e0} for lymphocytopenic effects

Subject	Dose	Total Lymphocytopenic Effect				CD8 Lymphocytopenic Effect			
		Cth	EC_{50}	AUEC	K_{e0}	Cth	EC_{50}	AUEC	K_{e0}
	mg	*ng/ml*	*ng/ml*	*%·h*	h^{-1}	*ng/ml*	*ng/ml*	*%·h*	h^{-1}
1	10	67.7	145.4	388	0.5827	29.5	106.3	720	0.3679
2	10	43.4	92.8	407	0.2680	25.9	96.0	474	0.4285
3	10	60.4	113.4	297	0.2879	45.1	105.6	390	0.2811
4	10	44.9	70.0	549	0.1965	46.6	69.0	576	0.2297
5	10	36.5	115.1	320	0.3749	42.3	150.2	235	0.4191
Mean (n = 5)		50.6	107.4	392	0.3420	37.8	105.4	479	0.3453
S.D.		12.9	28.1	99	0.1488	9.5	29.2	183	0.0871
6	30	112.2	146.8	546	0.1668	111.6	145.0	556	0.1659
7	30	77.1	197.3	397	0.4402	79.9	181.7	414	0.3673
8	30	100.6	186.0	341	0.2539	110.1	180.9	357	0.2984
9	30	121.1	159.2	519	0.2320	117.6	177.5	476	0.2882
10	30	94.9	213.0	523	0.4281	131.2	225.7	450	0.3895
Mean (n = 5)		101.2	180.5	456	0.3042	110.1	182.2	451	0.3019
S.D.		16.8	27.2	90	0.1230	18.8	28.7	74	0.0875
11	60	180.3	223.2	571	0.2296	181.2	213.8	637	0.2387
12	60	111.1	165.9	599	0.2873	117.1	167.8	576	0.2526
13*	60								
14	60	127.3	185.1	603	0.1868	140.4	181.6	623	0.1968
15	60	135.8	231.3	568	0.1739	161.3	313.4	316	0.1578
Mean (n = 4)		138.6	201.4	585	0.2194	150.0	219.1	538	0.2115
S.D.		29.6	31.1	18	0.0511	27.5	65.7	150	0.0429

* Effect data of subject 13 were insufficient for parameter estimations.

TABLE 2

Pharmacodynamic parameters and K_{e0} from the mean effect data of the three dosage groups

	Dosage					
	10 mg		30 mg		60 mg	
	Total[a]	Free	Total	Free	Total	Free
Total lymphocytopenic effect:						
Cth (ng/ml)	48.4	5.8	100.5	18.0	131.6	30.2
EC_{50} (ng/ml)	108.5	13.5	190.8	37.6	199.5	46.4
AUEC (%·h)	381	382	438	438	626	631
k_{e0} (h^{-1})	0.3234	0.2383	0.3367	0.2284	0.2284	0.1439
CD8 lymphocytopenic effect:						
Cth (ng/ml)	43.3	3.8	111.7	20.6	150.0	35.1
EC_{50} (ng/ml)	101.3	14.2	186.1	36.6	208.1	51.1
AUEC (%·h)	428	444	431	432	586	584
k_{e0} (h^{-1})	0.3360	0.3013	0.3305	0.2158	0.2519	0.1692
Cellular MLC effect:						
Cth (ng/ml)	27.3	3.5	98.1	17.5	123.9	29.4
EC_{50} (ng/ml)	218.1	25.6	294.1	59.1	218.9	52.3
AUEC (%·h)	264	265	277	278	580	579
k_{e0} (h^{-1})	0.2941	0.2083	0.3149	0.2111	0.2764	0.1679

[a] Total/free prednisolone.

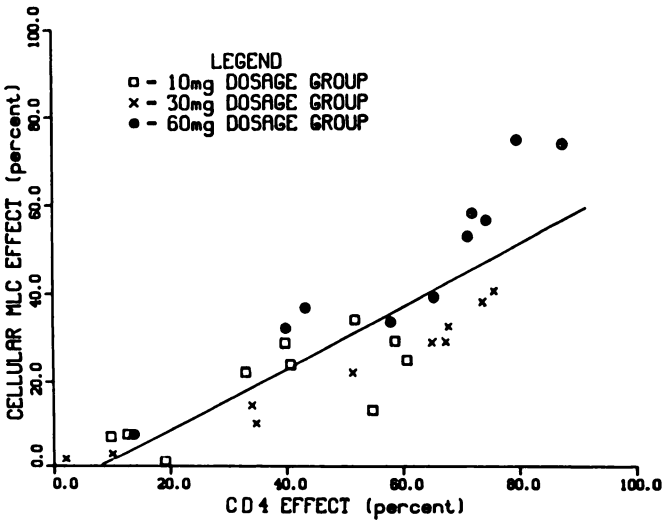

Fig. 3. Correlation plot of the cellular MLC effect *vs.* the CD4 lymphocytopenic effect; mean data of the three dosage groups.

cytopenic effect have a comparable time course, but that the latter is less sensitive toward prednisolone. It has been demonstrated that T-helper cells belong to the subset that is recognized by CD4, whereas T-suppressor cells are in the CD8 subset (Reinherz and Schlossman, 1980; Meuer *et al.*, 1982). Therefore, the diminished number of circulating CD4 lymphocytes as such and the decrease of the CD4/CD8 ratio in peripheral blood would be of importance for the immunosuppressive effect of prednisolone. This could be expected at least for those immunological reactions that take place outside the lymphoid organs so that lymphocytes have to circulate in order to arrive at their targets (ten Berge *et al.*, 1984). As standard numbers of remaining lymphocytes were added to the cultures, and no prednisolone or other dissolved compounds from the plasma of the concerning subjects were present, the reduced proliferative response (cellular MLC effect) seems due to the relative diminution of CD4 cells in the cultures.

The remarkable correlation between cellular MLC effect and CD4 lymphocytopenic effect may support this association (fig. 3). As k_{e0} and Cth of the total lymphocytopenic effect and the CD4 subset are similar, it can be understood that total lymphocyte counts is a good predictor for the cellular MLC response. Assuming that the latter *in vitro* response may be considered as a measure for *in vivo* immunoreactivity, total lymphocyte counts may be a simple and useful measure to monitor the indirect immunosuppressive effect of prednisolone.

Prednisolone may also directly suppress lymphocyte proliferation (Cupps and Fauci, 1982). This can be observed in MLC cultures, provided prednisolone is present at the time of antigen stimulation. This type of suppression is what we called the plasma MLC effect. As only 20% of plasma was added to the culture medium, a rather small effect was observed even in the 60-mg dosage group. The time course of the plasma MLC effect was apparantly concurrent with the time course of the prednisolone serum concentration, as no hysteresis is observed in figure 5. This is in agreement with the findings of Frey *et al.* (1982), who used a similar MLC setup as a tool for monitoring immunosuppressive therapy with prednisolone. In their approach, the inhibitory capacity of plasma, obtained after prednisolone administration, on the proliferation of the mixed lymphocytes of two donors was measured by adding 50% of plasma to the cultures. They found that the time course of their effect data was concurrent with the time course of total and free prednisolone serum concentrations even after i.v. administration. It should be noticed that their effect data *vs.* prednisolone serum concentrations could be fitted on a simple hyperbolic concentration-effect relationship in the case of patients who received chronic prednisolone treatment (Frey *et al.*, 1982). In the case of healthy subjects, they use a sigmoid concentration effect relation (Frey and Frey, 1984; Frey *et al.*, 1984). Although they give no explanation for this discrepancy it seems likely to be due to the difference in endogenous hydrocortisone levels between the patients, whose adrenal function must have been suppressed almost completely (Swartz and Dluhy, 1978) and healthy subjects. So the exponent in their equation has the same function as the threshold in ours. An advantage of the threshold E_{max} concentration-effect relation could be that it is applicable in both cases, yielding a different value for the threshold. It is remarkable that we find a similar threshold concentration (total and free prednisolone) for the plasma MLC effect as for the other effects of the 60-mg dosage

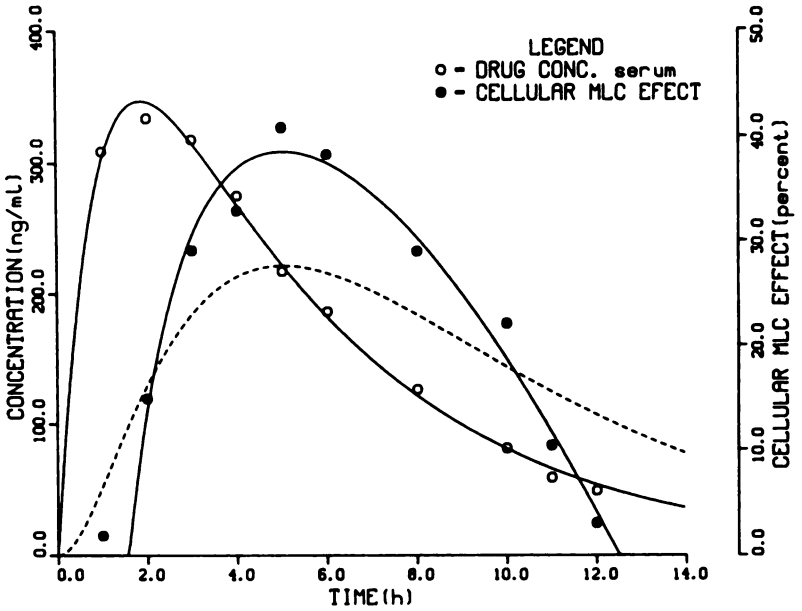

Fig. 4. Time course of the prednisolone serum concentration (CONC) and the cellular MLC effect; mean data of the 30-mg dosage group. Drawn lines represent fits to pharmacokinetic and integrated model; dashed line gives the time course of Ce.

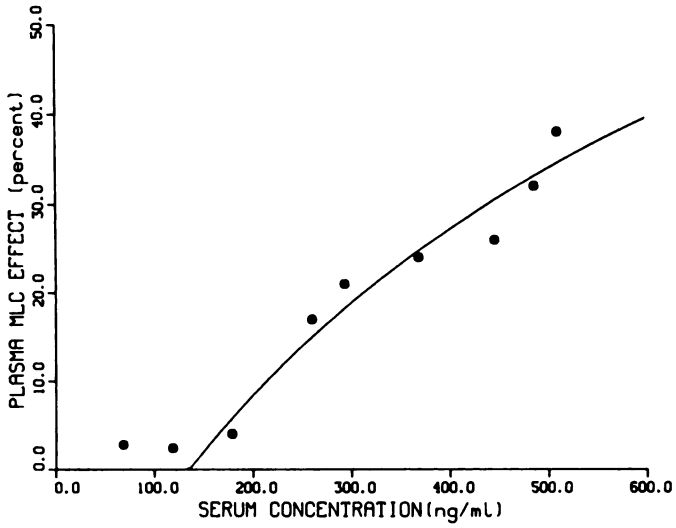

Fig. 5. Plasma MLC effect plotted *vs.* prednisolone serum concentration; mean data of the 60-mg dosage group (drawn line represents fit to equation 2).

group. In view of the different time courses of the direct effect and the lymphocytopenic effect, one may expect that the two effects are supplementary with respect to the immunosuppression by prednisolone. During the first 2 or 3 h after prednisolone administration the direct effect will be most important, whereas in the subsequent hours the contribution of the lymphocytopenia will increase. Estimating the relative contribution of the different effects to the overall immunosuppression by prednisolone is the subject of a study that is in preparation.

Taking into account the results of the present study, as well as the findings of Frey *et al.* (1982, 1984) about the plasma MLC effect, it can be concluded that, after the administration of a prednisolone dose of 60 mg, both effects are extinguished after about 14 h. Increasing the dose above 60 mg will hardly enhance either effect, due to the approximation of E_{max}. The latter two findings suggest that the administration of *e.g.* 30 mg of prednisolone every 12 h may be more effective than 60 mg daily. Smaller doses also seem favorable as they cause less depletion of endogenous hydrocortisone. However, the consequences of the increasing depletion of hydrocortisone, that is anticipated during multiple dosing of prednisolone, require further investigations.

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