Pharmacokinetic/Pharmacodynamic Evaluation of Deflazacort in Comparison to Methylprednisolone and Prednisolone

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Purpose. The pharmacokinetics and pharmacodynamics of deflazacort after oral administration (30 mg) to healthy volunteers were determined and compared with those of 20 mg of methylprednisolone and 25 mg of prednisolone. ***Methods.*** Methylprednisolone, prednisolone and the active metabolite of deflazacort, 21-desacetyldeflazacort, were measured in plasma using HPLC. For the assessment of pharmacodynamics, differential white blood cell counts were obtained over 24 hours. An integrated pharmacokinetic-pharmacodynamic (PK-PD) model was applied to link corticosteroid concentrations to the effect on lymphocytes and granulocytes. ***Results.*** Deflazacort is an inactive prodrug which is converted rapidly to the active metabolite 21-desacetyldeflazacort. Maximum concentrations of 21-desacetyldeflazacort averaged 116 ng/ml and were observed after 1.3 h. The average area under the curve was 280 ng/ml · h, and the terminal half-life was 1.3 h. 21-Desacetyldeflazacort was cleared significantly faster than both methylprednisolone and prednisolone. The PK-PD-model was suitable to describe time course and magnitude of the observed effects. The results were consistent with reported values for glucocorticoid receptor binding affinities for the investigated compounds. ***Conclusions.*** Due to the short pharmacokinetic half-life of its active metabolite, pharmacodynamic effects of deflazacort are of shorter duration than those of methylprednisolone and prednisolone. The PK-PD model allows good prediction of pharmacodynamic effects based on pharmacokinetic and receptor binding data.

KEY WORDS: pharmacokinetics; pharmacodynamics; corticosteroids; metabolites; prodrug.

INTRODUCTION

Deflazacort is a methyloxazoline derivative of prednisolone (Fig. 1) and has been proposed to have major advantages over other corticosteroids (1). It represents an inactive prodrug which is rapidly converted in the body to its active alcohol metabolite, 21-desacetyldeflazacort (Fig. 1). Deflazacort is rapidly absorbed and hydrolyzed after oral administration; average peak plasma concentrations of the active metabolite have been reported to be reached in 2 hours (2). The plasma half life of 21-desacetyldeflazacort was found to be 1.9 hours, and the plasma protein binding was 40%.

It was the purpose of the present study to evaluate the pharmacokinetics and pharmacodynamics of deflazacort after oral administration (30 mg) and compare the results with those after oral administration of 20 mg of methylprednisolone and 25 mg of prednisolone.

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METHODS

Subjects

The study was performed in a randomized, cross-over design in eight healthy subjects (5 male, 3 female). The average age was 29 years, the average weight 71 kg. The study was approved by an appropriate review board and subjects were fully informed of the protocol and intent of the study.

Study Protocol

The drugs were administered at 8 a.m. after an overnight fast. On the day before the study, subjects were asked to drink 1 L of fluids between 6 p.m. and 10 p.m. to standardize baseline conditions. The tablets (30 mg deflazacort in a single tablet, 25 mg prednisolone in 5 tablets of 5 mg, and 20 mg methylprednisolone in 5 tablets of 4 mg) were swallowed with 100 ml tap water. A standardized meal was served 4 hours after drug administration. The subjects were asked to restrain from alcohol, caffeine and nicotine use during the study period, and they were not taking any other medication.

Blood samples were obtained prior to drug administration and after 15, 30, 45, 60, 90, 120 and 150 minutes as well as 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20 and 24 hours after drug administration. The samples were centrifuged; the harvested plasma was frozen immediately and stored at −20°C until analyzed.

For the pharmacodynamic evaluation, differential white blood cell counts were performed before drug administration and after 2, 4, 6, 8, 10, 12, 14, 16, 20 and 24 hours.

Analytical Method

A sensitive, specific and precise HPLC method was adapted and used for the determination of prednisolone and methylprednisolone in human plasma (3). Dexamethasone was used as internal standard. Human plasma (1 ml) was extracted with 12 ml methylene chloride. The organic phase was washed with 2 ml of 0.1 N sodium hydroxide solution and 1 ml of water. Anhydrous sodium sulfate (1 g) was used to remove water. The extract was dried under nitrogen and reconstituted with mobile phase (hexane:methylene chloride:ethanol:glacial acetic acid 260:690:34:20). The solution was injected onto a silica column (Sperisorb 3 μ, 15 × 4.6 cm, Deside Ind. Est., Queensferry, UK) using a LDC Constametric IIIG pump to give a flow rate of 0.75 ml/min. An autoinjector (WISP 710B, Waters Associates) was used for injection. UV detection was performed at 254 nm using a LDC/Milton Roy spectromonitor. Chromatograms were obtained using a Hewlett Packard HP 3394A integrator. Representative retention times were dexamethasone (5.1 min), hydrocortisone (6.3 min), prednisolone (7.7 min) and methylprednisolone (7.4 min). The assay was precise, accurate and reproducible. The limit of quantification was less than 10 ng/ml. The coefficient of variation for accuracy and preci-

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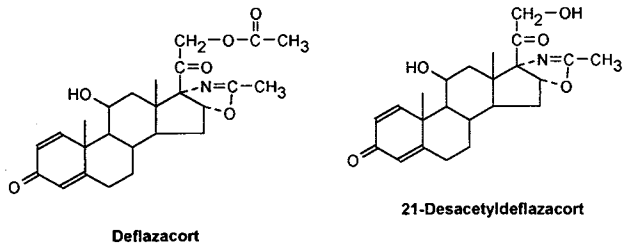

Fig. 1. Chemical structures of deflazacort and its active metabolite 21-desacetyldeflazacort.

sion was less than 10% in the concentration range of 10–1000 ng/ml.

21-Desacetyldeflazacort was extracted from buffered plasma samples to which an internal standard (betamethasone) had been added, by solid phase extraction on 1 ml cyanopropyl columns. Extracts were reduced to dryness and reconstituted in HPLC mobile phase. Reconstituted extracts were then chromatographed on a 25 cm 5 μm ODS HPLC column by gradient elution with mixtures of pH 3.0 phosphate buffer, acetonitrile and tetrahydrofuran. Detection was by UV spectroscopy at 254 nm. The limit of quantification of 21-desacetyldeflazacort was 10 ng/ml. However, under the described conditions it was not possible to simultaneously measure hydrocortisone; hence, no hydrocortisone measurements were performed after administration of deflazacort.

Pharmacokinetic Data Analysis

Pharmacokinetic analysis was performed using noncompartmental and compartmental methods. Area under the plasma concentration-time curve (AUC) and area under the first moment curve (AUMC) were calculated using the trapezoidal rule. The mean residence time (MRT) was calculated as AUMC/AUC. Maximum plasma concentration (C_{max}) and time of maximum plasma concentration (t_{max}) were obtained directly from the experimental data. Oral clearance (CL/F) was calculated as dose (D) divided by AUC where F stands for the bioavailable fraction of the dose. In the case of deflazacort, F also includes the fraction of the dose converted to the measured active metabolite. The apparent volume of distribution (Vd/F) was calculated as the product of CL/F and $t_{1/2/0.693}$.

The plasma concentrations were converted to their respective unbound concentrations. For methylprednisolone and deflazacort, no concentration dependence in protein binding has been reported so that the constant factors of 0.23 for methylprednisolone (4,5) and 0.6 for deflazacort (2) could be used. The concentration-dependent free concentrations of prednisolone were calculated according to Frey (6,7) using the Microsoft EXCEL Solver subroutine. For the PK/PD-analysis a compartmental pharmacokinetic analysis was performed using RSTRIP (MicroMath, Salt Lake City, Utah). The free plasma concentrations were fitted to a one compartment body model with first order absorption, according to the equation

$$Cp_f = A \cdot (e^{-k_e \cdot t} - e^{-k_a \cdot t}) \quad (1)$$

with

$$A = \frac{k_a \cdot F \cdot f_u \cdot D}{Vd \cdot (k_a - k_e)}$$

where k_a and k_e are the rate constants for absorption and elimination, respectively, and f_u is the unbound fraction in plasma.

Pharmacodynamic Data Analysis

Pharmacodynamic parameters evaluated included the number of lymphocytes, monocytes and granulocytes. These parameters were measured using standard techniques over 24 hours. As a cumulative measure of pharmacodynamic activity on the blood cells, the area under the effect-time curve (AUC_E) was calculated using the trapezoidal rule and the pre-dose value as a baseline for each individual.

PK/PD-Model

A pharmacokinetic-pharmacodynamic model derived earlier (8,9) was utilized to relate the measured drug concentrations to the respective effect. It was assumed that under steady state conditions the number of blood cells are constant due to equal degree of influx of cells from the extravascular space into the blood and efflux of cells out of the vascular space. If the influx is assumed to follow zero-order kinetics with a zero-order rate constant (k_{in}), and the efflux is assumed to follow first-order kinetics with a first-order rate constant (k_{out}), then the rate of change of the number of blood cells, N, can be quantified as

$$\frac{dN}{dt} = k_{in} - k_{out} \cdot N \quad (2)$$

At steady state, this difference is zero, since influx and efflux are of the same magnitude. It could be shown (8,9) that excellent predictions are possible if the steroid effect on the number of blood cells is assumed to take place by modifying the influx and, hence, changing k_{in}. In the case of the lymphocytes, k_{in} is decreased causing a depletion of the number of cells in the blood. Since corticosteroids act via receptor activation, the E_{max}-model is the most appropriate pharmacodynamic model to relate free steroid concentrations on the effect of influx. Therefore, the number of lymphocytes will be affected by corticosteroids according to

$$\frac{dN}{dt} = k_{in} \cdot \left(1 - \frac{E_{max} \cdot Cp_f}{E_{50} + Cp_f}\right) - k_{out} \cdot N \quad (3)$$

If the number of lymphocytes are converted to percent of pre-dose numbers, then by definition the number of cells at time zero is 100%. Since this value is observed at steady state, it follows that $k_{in} = 100 \cdot k_{out}$. Furthermore, it can be assumed that the value k_{in}, k_{out} and E_{max} are identical for the three treatments for a given subject and independent of the steroid used. However, E_{50} is a function of the intrinsic potency of the respective steroid induced by its receptor binding affinity. Hence, it is possible with the use of nonlinear regression programs which allow the use of differential equations (SCIENTIST, MicroMath, Salt Lake City, UT) to simultaneously fit all data for a given parameter using the respective pharmacokinetic and pharmacodynamic results.

This procedure was performed for each individual subject as well as for the overall means.

In the case of the granulocytes, the exact same model was applied; however, since the effect of the steroids is an increase of granulocytes in blood, the E_{max}-term in equation 3 was added rather than subtracted.

RESULTS

Pharmacokinetics

The results of the non-compartmental pharmacokinetic analysis are listed in Table I. The mean plasma concentrations for the three steroids are shown in Fig. 2A. As has been reported before (10,11), prednisolone shows a lower oral clearance (CL/F) at this dose than methylprednisolone (14 L/h vs. 30 L/h) so that higher prednisolone levels are observed. However, of the three steroids investigated, 21-desacetyldeflazacort shows by far the highest oral clearance (114 L/h) assuming a complete conversion of deflazacort to its active metabolite.

The apparent volume of distribution (Vd/F) is lower for prednisolone (69 L) than for methylprednisolone (106 L) and 21-desacetyldeflazacort (204 L) assuming quantitative prodrug conversion. The half-life is the shortest for 21-desacetyldeflazacort (1.3 h) followed by methylprednisolone (2.4 h) and prednisolone (3.6 h). Similar half-lives of 2.8 hours have been reported before for methylprednisolone (12); for prednisolone reported half-lives varied from 2.4 to 3.7 hours (11,13). The mean residence times (MRT) of the three compounds confirms the observed ranking order: 21-desacetyldeflazacort has a MRT of only 2.5 h whereas methylprednisolone (MRT 4.1 h) and prednisolone (MRT 4.9 h) show longer values.

The oral absorption was found to be fastest with deflazacort (t_{max} 1.3 h) and prednisolone (t_{max} 1.6 h) in comparison with methylprednisolone (t_{max} 2.7 h). After administration of the investigated doses, the highest peak concentrations were observed for 25 mg prednisolone with a C_{max} 362 ng/ml vs. a C_{max} of 182 ng/ml after 20 mg of methylprednisolone and a C_{max} of 116 ng/ml for the highest dose of 30 mg deflazacort.

When the data was converted to the respective unbound concentration in plasma (Fig. 2B), the half life for prednisolone significantly shortened due to the well-known nonlinear protein binding of prednisolone (6,7,9). The calculated half-life of free prednisolone was 2.3 h compared to 3.6 h for the total concentrations. The free concentrations were fitted to Eq. 1 using a one-compartment body model. The resulting estimates for A, k_a and k_e were 139.8 ng/ml, 1.07 h^{-1} and 0.307 h^{-1} for unbound prednisolone, 201.5 ng/ml, 0.55 h^{-1} and 0.366 h^{-1} for unbound methylprednisolone and 150.2 ng/ml, 1.22 h^{-1} and 0.493 h^{-1} for unbound 21-desacetyldeflazacort, respectively. These parameters were then used as estimates for the integrated PK-PD-data evaluation.

Pharmacodynamics

The effects on granulocytes and lymphocytes was marked. For both cell types, the lower dose of methylprednisolone produced a significantly stronger effect than the other two steroids. The effect of prednisolone (25 mg) was similar to methylprednisolone (20 mg) in its time course, but lower in magnitude; the effects of deflazacort were initially of comparable magnitude, but of shorter duration and disappeared at a significantly faster rate. Comparison of the cumulative effects of the three treatments, expressed as the respective area under the effect-time curves shows significantly stronger effects for methylprednisolone in the dose given than for the two other steroids, which are not significantly different with respect to their cumulative effect over 24 hours. The average AUC_E for methylprednisolone, prednisolone and deflazacort were 900 ± 261, 571 ± 406 and 494 ± 353 % · h, respectively, for the lymphocyte depression; and 693 ± 204, 449 + 312 and 399 ± 292 % · h, respectively, for the increase in the number of granulocytes.

PK/PD-Model

The data was furthermore subjected to the integrated PK-PD analysis as described above. For the two parameters lymphocytes and granulocytes the data was fitted for each individual simultaneously for the three steroids with a single value for E_{max} and k_{out}, but different E_{50} values for each steroid. Furthermore, the mean data was subjected to the same procedure. With this approach, it was possible to obtain reasonable agreement of experimental data and the respective fitted curves for both granulocytes (Fig. 3) and lymphocytes (Fig. 4). The correlation coefficients were 0.979 for the lymphocytes and 0.969 for the granulocytes.

Tab. II lists the PK/PD-parameters for the effects on granulocytes and lymphocytes. Analysis of the average

Table I. Mean Pharmacokinetic Data (±S.D.) Obtained from Noncompartmental Pharmacokinetic Analysis

	Methylprednisolone	Prednisolone	21-Desacetyl-deflazacort
D [mg]	20	25	30 (deflazacort)
AUC [ng/ml · h]	800 ± 386	1942 ± 471	280 ± 85
AUMC [ng/ml · h^2]	3340 ± 1708	9783 ± 3420	822 ± 305
$t_{1/2}$ [h]	2.4 ± 0.9	3.6 ± 0.8	1.3 ± 0.4
MRT [h]	4.1 ± 1.0	4.9 ± 1.0	2.5 ± 0.5
C_{max} [ng/ml]	182 ± 97	362 ± 72	116 ± 40
t_{max} [h]	2.7 ± 1.2	1.6 ± 0.7	1.3 ± 0.5
CL/F [l/h]	30 ± 13	14 ± 3	114 ± 27
Vd/F [l]	106 ± 63	69 ± 16	204 ± 84

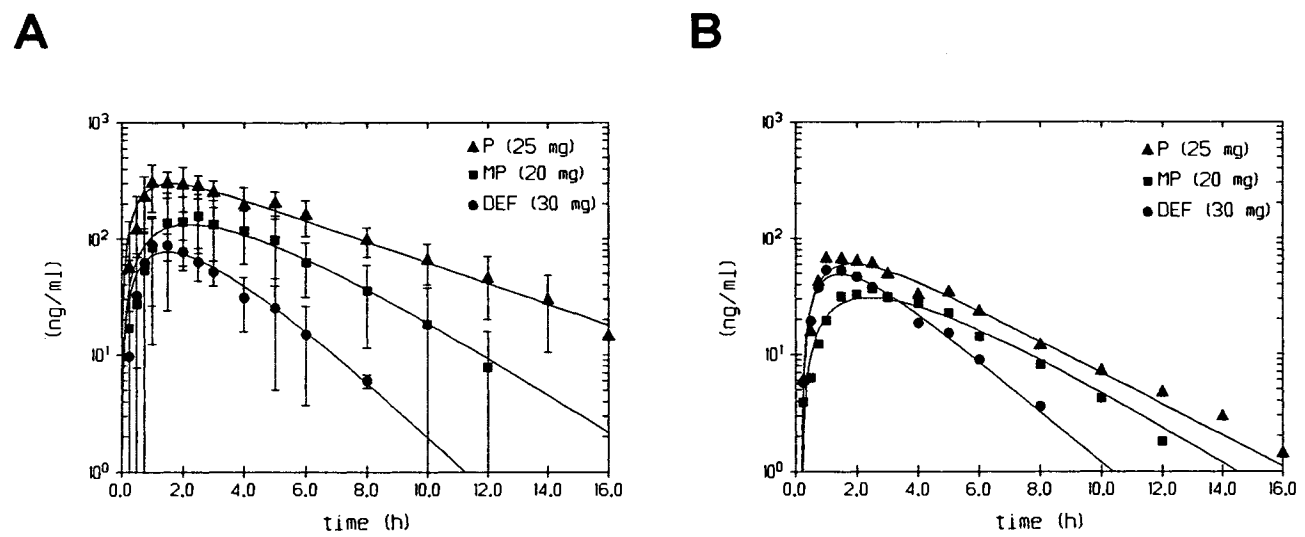

Fig. 2. A. Mean plasma concentration (±S.D.) after oral administration of 20 mg methylprednisolone (MP) and 25 mg prednisolone (P) as well as mean plasma concentration of 21-desacetyldeflazacort (DEF) after oral administration of 30 mg deflazacort. B. Mean free (unbound) concentration of methylprednisolone (MP), prednisolone (P) and 21-desacetyldeflazacort (DEF) for the same data.

pharmacodynamic data gave comparable results to the mean of the individual data, although the between-subject variability was considerable. For this reason, Tab. II also reports the median of the individual data which is in excellent agreement with the parameters derived from the average data.

DISCUSSION

The presented results give another example of the usefulness of PK-PD-modeling for corticosteroids as it has been shown before (8,9). Since for all corticosteroids the post-receptor events are thought to be identical, the inclusion of receptor binding data (14,15) can serve as an important addition to the classical effect compartment approach. When compared with dexamethasone (relative binding affinity 100%), the relative binding affinities for methylprednisolone, prednisolone and 21-desacetyldeflazacort are 42%, 16% and 29%, respectively. Although the absolute numbers for E_{50} parameters determined from PK-PD modeling and in-vitro receptor binding IC_{50} are not identical, the relative potency order is in good agreement: methylprednisolone shows stronger intrinsic pharmacodynamic potency than 21-desacetyldeflazacort and prednisolone. There is a reasonable correlation between these two values (r^2 = 0.73 for granulocytes and 0.78 for lymphocytes). In the case of deflazacort, the model is clearly able to take into account the significantly different pharmacokinetic properties. 21-Desacetyldeflazacort is cleared at a much higher rate than the other two steroids which leads to a faster rate of disappearance of the pharmacodynamic effect. Hence, the PK-PD-model separates the pharmacokinetic and pharmacody-

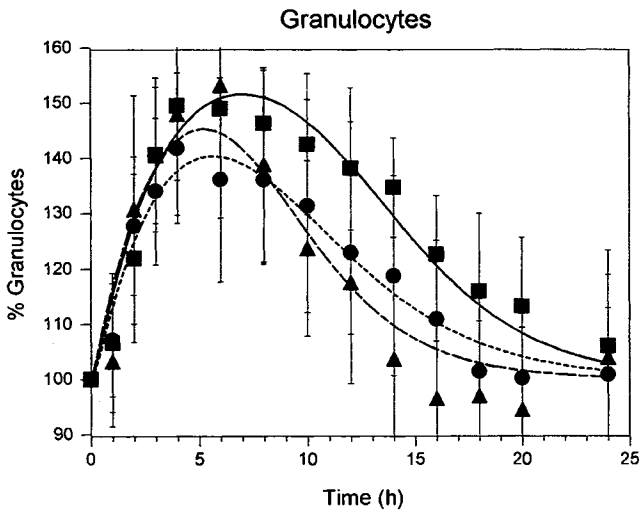

Fig. 3. Pharmacokinetic-pharmacodynamic data fit for the effect on the number of granulocytes using the described PK-PD model. The points are the experimental data (±S.D.), the lines are the model-fitted curves. Symbols in the overlay correspond to methylprednisolone (■), prednisolone (●) and deflazacort (▲).

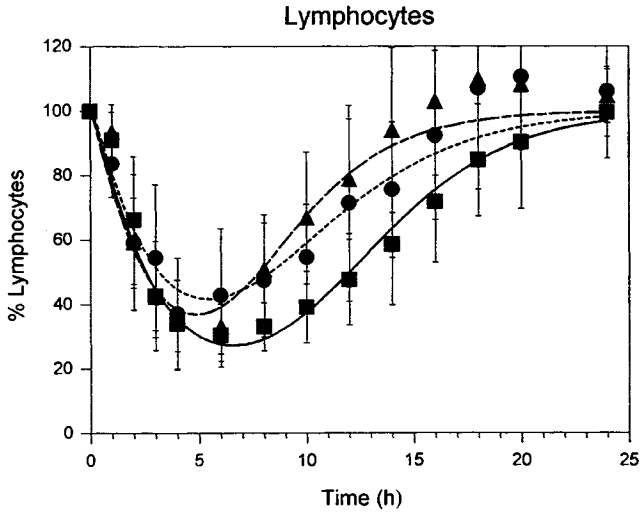

Fig. 4. Pharmacokinetic-pharmacodynamic data fit for the effect on the number of lymphocytes using the described PK-PD model. The points are the experimental data (±S.D.), the lines are the model-fitted curves. Symbols in the overlay correspond to methylprednisolone (■), prednisolone (●) and deflazacort (▲).

Table II. PK/PD Parameters (means ± S.D.) from Curve Fits of Average and Individual Data

	Average data	Individual data	Median from individual data
Granulocytes			
E_{max}	0.68 ± 0.12	0.69 ± 0.21	0.70
k_{out} [h^{-1}]	0.35 ± 0.07	0.43 ± 0.11	0.41
$E_{50(MP)}$ [ng/ml]	3.6 ± 2.0	5.6 ± 5.6	4.4
$E_{50(P)}$ [ng/ml]	16.7 ± 6.8	15.0 ± 6.0	14.9
$E_{50(DEF)}$ [ng/ml]	5.6 ± 3.0	9.8 ± 11.0	6.7
Lymphocytes			
E_{max}	1.00 ± 0.19	0.86 ± 0.15	0.92
k_{out} [h^{-1}]	0.37 ± 0.07	0.43 ± 0.14	0.41
$E_{50(MP)}$ [ng/ml]	4.8 ± 2.7	3.2 ± 3.2	3.6
$E_{50(P)}$ [ng/ml]	18.6 ± 8.0	15.1 ± 15.7	10.1
$E_{50(DEF)}$ [ng/ml]	7.7 ± 4.2	5.9 ± 4.3	5.6

namic contributions to the overall effect. For example, the model predicts that although 21-desacetyldeflazacort has a higher receptor affinity than prednisolone, its overall potency will not be higher, because prednisolone has a longer pharmacokinetic residence time and can exert its effects for a longer time.

The presented model varies from that proposed earlier (16,17) for a study where there was not sufficient data to characterize the onset of action in detail and a lag-time was used to model the delay in initial response. The model used in this paper has been shown to be applicable with good results (7,8). However, both models are conceptually similar in that they allow the inclusion of receptor binding data to make predictions of the expected time-response curves based on pharmacokinetic data and in-vitro binding data (hard-link). Most significant for such a prediction is the fact, that a correlation has been shown between the easily quantifiable pharmacodynamic response on blood cells and the clinically observed empirical activity as quantified by commonly used equivalence doses (17).

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