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PREDNISOLONE BINDING TO ALBUMIN AND TRANSCORTIN IN THE PRESENCE OF CORTISOL*

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Abstract—The protein binding of prednisolone was assessed in a 5% albumin solution and in pooled human serum, alone and in the presence of various amounts of cortisol. Significant displacement of prednisolone from transcortin binding sites occurred with little or no change in transcortin binding capacity or affinity constant for prednisolone, suggesting competitive inhibition of prednisolone binding by cortisol. Little or no displacement of prednisolone from the protein binding sites on albumin occurred though a decrease in the number of binding sites (four vs two), and an increase in the affinity constant for the albumin–prednisolone interaction (4.32×10^2 vs 9.43×10^2 M^{-1}) occurred in the presence of cortisol. Allosteric or conformational changes may occur in albumin structure in the presence of cortisol. These alterations have no effect on the fraction of prednisolone bound to albumin.

Prednisolone is a unique synthetic glucocorticoid in that it competes with endogenous cortisol for protein binding sites on serum proteins [1]. This competition might be anticipated based on structural considerations since these steroid molecules are identical, with the exception of the presence of the 1-2 double bond in the A-ring of the prednisolone nucleus. Transcortin, an α_1-glycoprotein, has a very high affinity but low capacity for binding these steroids. This protein is easily saturated when serum concentrations of these compounds are increased, resulting in increases in the free fraction of these steroids. Albumin, the other protein responsible for steroid binding, has a low affinity but a high capacity for associating with these steroids [2]. The net result of these steroid–protein interactions is a concentration-dependent binding pattern, with each of these steroids being bound more than 90% at low concentrations and approximately 55% at high steroid concentrations [2].

The competition between cortisol and prednisolone is of interest in that protein binding has been implicated as a major factor contributing to the nonlinearity in the distribution and clearance of prednisolone *in vivo* [3]. Such competition may alter the pharmacokinetics of these agents through the production of changes in the free fraction of these compounds in blood. In addition, this competition has pharmacologic implications in that only the unbound corticosteroid in plasma appears to be therapeutically active [4].

The purpose of this study was to examine the competition between cortisol and prednisolone for binding sites on albumin alone and on albumin and transcortin in plasma.

MATERIALS AND METHODS

Binding studies. The protein binding of prednisolone was examined in a 5% solution of Fraction V human serum albumin (HSA) prepared in isotonic phosphate buffer (pH 7.4). Prednisolone was added to twenty 10-ml aliquots of this solution to obtain concentrations in the 25–250,000 ng/ml range. Portions of each aliquot were used to examine the protein binding of prednisolone alone and in the presence of 25, 250, 2500, 25,000 and 250,000 ng/ml of cortisol.

Analogous studies were performed using pooled serum that was charcoal treated to remove endogenous cortisol. The absence of cortisol was assured by analysis using the high performance liquid chromatography (h.p.l.c.) technique of Rose and Jusko [5].

The protein binding determinations were performed using equilibrium dialysis at 37°. Binding was measured using [6,7-^{3}H(N)]prednisolone alone or in conjunction with [4-^{14}C]cortisol in studies where cortisol was present as a competitor. The specific activities of these compounds were 53 and 55 Ci/mmole. Prior to use, the radiolabeled prednisolone and cortisol were purified by h.p.l.c., using a microparticulate silica gel column and a mobile phase consisting of 3.5% methanol in methylene chloride.

To a 0.8-ml aliquot of sample, trace quantities of radioactivity were added. The sample was then dialyzed against an equal volume of isotonic phosphate buffer (pH 7.4) for a period of 16 hr which was shown in preliminary studies to afford stability and attainment of equilibrium. Following dialysis, 0.5 ml of serum and buffer were removed from the cells and placed into scintillation vials. Water (2.5 ml) and liquid scintillation mixture (10 ml) were

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added, and each vial was capped and then shaken to form a stiff gel. The samples were then counted on a Packard scintillation counter using energy windows preset for ^{3}H–^{14}C dual label counting. The samples were counted for 20,000 counts (in each channel) or 20 min, whichever occurred first. The counts per minute for each isotope were converted to disintegrations per minute using the external standards ratio technique [6]. Free and bound concentrations of the steroids were then calculated from the dpm values and the initial total steroid concentrations using methods similar to Behm and Wagner [7].

Data analysis. The data were evaluated through the use of the Rosenthal relationship [8]. The equation which describes the binding of prednisolone (with no cortisol present) in the albumin solution is:

$$D_{B,PN} = \frac{N_A \cdot P_A \cdot K_{A,PN} \cdot D_{F,PN}}{1 + K_{A,PN} \cdot D_{F,PN}} \quad (1)$$

where $D_{B,PN}$ is the molar concentration of bound prednisolone, $D_{F,PN}$ is the molar concentration of unbound (free) prednisolone, N_A is the number of binding sites on the albumin molecule, P_A is the molar albumin concentration, and $K_{A,PN}$ is the affinity constant for albumin–prednisolone.

In the presence of cortisol, competition between prednisolone and cortisol occurs and the binding equation for prednisolone is:

$$D_{B,PN} = \frac{N_A \cdot P_A \cdot K_{A,PN} \cdot D_{F,PN}}{1 + K_{A,PN} \cdot D_{F,PN} + K_{A,C} \cdot D_{F,C}} \quad (2)$$

where $K_{A,C}$ and $D_{F,C}$ are the affinity constant for albumin–cortisol binding and the molar concentration of unbound cortisol.

Examination of the binding of prednisolone in pooled serum is more complex because transcortin is present. As a result, an additional term is needed in equations 1 and 2 to reflect the binding of prednisolone and cortisol to transcortin. Incorporation of this term into equation 1 yields equation 3:

$$D_{B,PN} = \frac{N_A \cdot P_A \cdot K_{A,PN} \cdot D_{F,PN}}{1 + K_{A,PN} \cdot D_{F,PN}} + \frac{N_T \cdot P_T \cdot K_{T,PN} \cdot D_{F,PN}}{1 + K_{T,PN} \cdot D_{F,PN}} \quad (3)$$

where N_T, P_T and $K_{T,PN}$ are the number of binding sites on the transcortin molecule, the serum transcortin concentration, and the affinity constant for the binding of prednisolone to transcortin. Equation 3 applies only in the absence of cortisol.

Similar inclusion of a transcortin binding term into equation 2 to reflect prednisolone serum binding in the presence of cortisol results in equation 4:

$$D_{B,PN} = \frac{N_A \cdot P_A \cdot K_{A,PN} \cdot D_{F,PN}}{1 + K_{A,PN} \cdot D_{F,PN} + K_{A,C} \cdot D_{F,C}} + \frac{N_T \cdot P_T \cdot K_{T,PN} \cdot D_{F,PN}}{1 + K_{T,PN} \cdot D_{F,PN} + K_{T,C} \cdot D_{F,C}} \quad (4)$$

where $K_{T,C}$ is the affinity constant for transcortin–cortisol binding.

The molar concentrations of bound prednisolone ($D_{B,PN}$), free prednisolone ($D_{F,PN}$) and free cortisol ($D_{F,C}$) in the post-dialysis samples were calculated using the fractions of prednisolone and cortisol bound and the initial serum concentrations of prednisolone and cortisol [7]. Equations 1–4 were used to fit the binding data using a modified version of the NONLIN computer program [9]. The molar concentration of bound prednisolone was the dependent variable and $D_{F,PN}$ and $D_{F,C}$ served as independent variables. Albumin concentration (P_A) was measured chemically and employed as a constant. The following nonlinear least-squares estimates were provided as a function of the equation used:

(1) N_A and $K_{A,PN}$ using equation 1 describing prednisolone binding in the albumin solution in the absence of cortisol;

(2) N_A, $K_{A,PN}$, and $K_{A,C}$ using equation 2 describing prednisolone binding in the albumin solution with cortisol present as a competitor;

(3) N_A, $K_{A,PN}$, $N_T \cdot P_T$, and $K_{T,PN}$ using equation 3 describing prednisolone serum binding in the absence of cortisol;

(4) N_A, $K_{A,PN}$, $K_{A,C}$, $N_T \cdot P_T$, $K_{T,PN}$ and $K_{T,C}$ for equation 4 describing prednisolone serum binding with cortisol present as a competitor.

RESULTS

Albumin binding. Three-dimensional depiction of the fraction of prednisolone bound to albumin as a function of unbound prednisolone and cortisol concentrations is presented in Fig. 1. An extensive range of steroid concentrations was employed allowing visualization of the saturation in albumin binding at very large drug concentrations. The parameters N_A, $K_{A,PN}$ and $K_{A,C}$ which resulted from the fitting of this binding data to equation 2 are presented in Table 1. The affinity of the albumin molecule for binding prednisolone was 300 times greater than that observed for binding cortisol. The N_A and $K_{A,PN}$ values are presented for the binding of prednisolone in the absence of cortisol, for comparison. The pres-

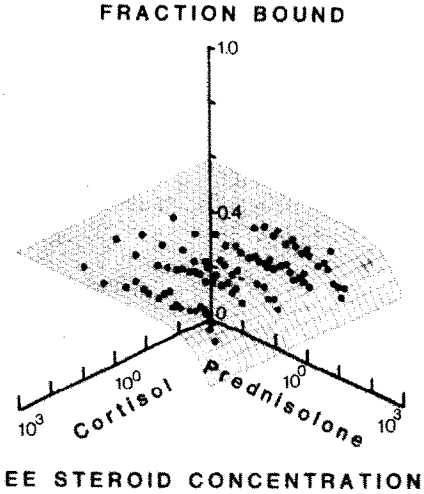

Fig. 1. Relationship between the fractional binding of prednisolone to human serum albumin (5%) and unbound concentrations of prednisolone and cortisol. Dots indicate experimental data, and surface denotes least-squares regression fit according to equation 2.

Table 1. Steroid protein binding parameters in a 5% albumin solution

Study	Prednisolone $K_{A,PN}$ ($\tilde{M}^{-1}$)	Prednisolone N_A	Cortisol $K_{A,C}$ ($\tilde{M}^{-1}$)
Prednisolone	4.32×10^2 (4.17×10^2–4.46×10^2)*	4.05 (3.92–4.20)	
Prednisolone and cortisol	9.43×10^2 (5.12×10^2–1.37×10^3)	2.00 (1.09–2.92)	2.79 (2.75–2.83)

* Numbers in parentheses indicate the 95% confidence intervals for the parameter.

ence of cortisol as a competitor of prednisolone binding resulted in a decrease in N_A from approximately 4 to 2. This was accompanied by an increase in the affinity of the prednisolone–albumin interaction.

Assessment of whether this change in N_A and $K_{A,PN}$ reflected the most suitable computer least-squares fitting was checked by characterizing the prednisolone–cortisol–albumin data with the original N_A and $K_{A,PN}$ values obtained in the absence of cortisol along with the generated $K_{A,C}$ value. A substantial difference in the sum of squared percent deviations of observed versus predicted $D_{B,PN}$ values (57,220 original vs 74,029) and a pronounced degree of systematic deviation in a plot analogous to Fig. 1 indicated that altered binding parameters were necessary to optimize the least-squares function.

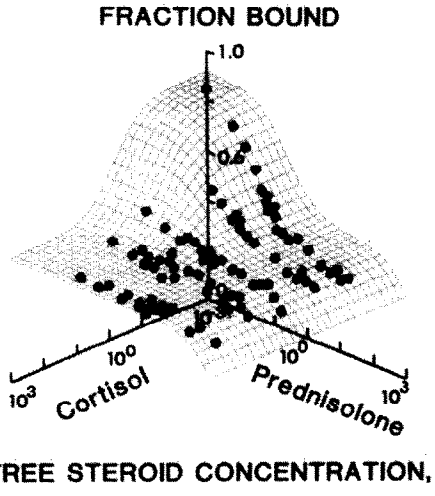

Fig. 2. Relationship between the fractional binding of prednisolone to pooled human plasma and unbound concentrations of prednisolone and cortisol. Dots indicate experimental data, and surface denotes least-squares regression fit according to equation 4.

Serum binding. A three-dimensional plot depicting the fraction of prednisolone bound as a function of unbound prednisolone and cortisol concentrations is presented in Fig. 2. Saturation in binding of prednisolone to both transcortin and albumin is evident. The binding parameters resulting from the fitting of these data to equation 4 are presented in Table 2 and are compared with the binding parameters obtained for prednisolone alone. The binding parameters reflecting prednisolone and cortisol binding to albumin are similar to those obtained in the albumin solution. Substantial displacement of prednisolone from protein binding sites on transcortin occurred with the addition of cortisol producing smaller values of fraction bound (Fig. 2), though little or no change occurred in the prednisolone–transcortin binding parameters (Table 2).

DISCUSSION

The protein binding parameters obtained for prednisolone in the albumin solution and in pooled serum agree well with the values obtained previously. We [10] measured the protein binding of prednisolone in normal human serum and obtained values of 6.2×10^{-7} M̃, 0.5×10^7 M̃$^{-1}$, and 12.1×10^2 M̃$^{-1}$ for $N_T \cdot P_T$, $K_{T,PN}$ and $K_{A,PN}$ which agree well with the values obtained in the present study. The value for $K_{A,PN}$ obtained in the previous study was an apparent value, however, since N_A was assumed to be equal to 1. The present study involved use of a wider range of steroid concentrations permitting more definitive calculation of N_A and a value of 2 was obtained in the presence of cortisol. This would reduce the $K_{A,PN}$

Table 2. Steroid protein binding parameters in pooled human serum*

Study	Prednisolone $N_T \cdot P_T$ (M̃)	Prednisolone $K_{T,PN}$ ($\tilde{M}^{-1}$)	Prednisolone $K_{A,PN}$ ($\tilde{M}^{-1}$)	Prednisolone N_A	Cortisol $K_{T,C}$ ($\tilde{M}^{-1}$)	Cortisol $K_{A,C}$ ($\tilde{M}^{-1}$)
Prednisolone	5.54×10^{-7}	1.50×10^7	4.34×10^2	3.98		
Prednisolone and cortisol	4.37×10^{-7}	2.47×10^7	9.43×10^2	2.00	1.01×10^7	2.79

* Albumin concentration measured as 5.22×10^{-4} M̃.

value obtained previously by one-half to $6.05 \times 10^2 \bar{M}^{-1}$.

The value of $K_{A,C}$ obtained in this study is much lower than the value obtained by Westphal [11] ($3 \times 10^3 \bar{M}^{-1}$) for cortisol binding to HSA in the absence of competitors. A possible explanation for this discrepancy will be presented below. In contrast the $K_{T,C}$ value agrees fairly well with the value of $3 \times 10^7 \bar{M}^{-1}$ obtained by Westphal [11].

Interaction between cortisol and prednisolone for protein sites on the albumin molecule is not evident from the binding plot (Fig. 1) but can be appreciated through comparison of the prednisolone–albumin binding parameters in the presence and absence of cortisol (Tables 1 and 2). In the presence of cortisol, a decrease in N_A and an increase in K_A were observed. The product $N_A \cdot K_A$, however, remained relatively constant (1.75×10^3 vs $1.89 \times 10^3 \bar{M}^{-1}$). This product directly co-determines $D_{B,PN}$ along with P_A and $D_{F,PN}$; (equation 2), and is probably responsible for the lack of systematic deviations in prednisolone binding with increases in cortisol concentrations. The increase in $K_{A,PN}$ that occurred in the presence of cortisol does not dramatically influence the magnitude of the denominator in equation 2 at the free concentration of prednisolone observed, due to the relatively low value of $K_{A,PN}$. The changes in N_A and $K_{A,PN}$ that occur in the presence of cortisol suggest a non-competitive interaction occurring at allosteric sites or other effects on the conformational structure of albumin, which may serve to change these binding parameters. Such a phenomenon is not unusual as Brunkhorst and Hess [12], in examining cortisol–albumin binding, noted increased values of the product $N_{A,C} \cdot K_{A,C}$ with decreases in albumin concentration. The number of cortisol binding sites/albumin molecule appears to be variable and partly dependent on steroid concentration and other conditions, as 1 to 20 is the $N_{A,C}$ given by Ballard [4]. These steroid interactions with albumin appear to have little significance *in vivo* in that the albumin bound fraction of prednisolone was not appreciably affected by the presence of cortisol (Fig. 1). The disparity between the $K_{A,C}$ values observed in the presence and absence of prednisolone suggests an interaction between prednisolone and albumin that noncompetitively alters the binding interaction between cortisol and albumin.

The presence of cortisol severely altered the fraction of prednisolone bound to transcortin (Fig. 2). The presence of cortisol in the serum did not result in appreciable alterations in the binding capacity of transcortin though a small increase in $K_{T,PN}$ was observed (Table 2). This increase in $K_{T,PN}$ may not be real, in that the fitting of the data obtained in pooled serum was more complex and did not permit error estimation for the binding parameters. The "goodness of fit" of the computer generated surface to the experimental data (Fig. 2) coupled with the agreement between the parameters obtained in the present study and those obtained previously (*vide supra*) lends credibilty to the binding parameters obtained in pooled serum. The lack of dramatic changes in the transcortin binding capacity and affinity for prednisolone in the presence of cortisol suggests competitive inhibition of prednisolone binding to transcortin by cortisol.

Comparison of the affinity constants of prednisolone and cortisol for albumin and transcortin (Tables 1 and 2) indicates that prednisolone binding is approximately 2.5 times stronger to transcortin and more than 300 times stronger to albumin. These differences in binding affinities suggest that the presence of the 1–2 double bond in the A-ring of the prednisolone molecule sterically favors the binding of prednisolone to each of these proteins.

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