Disposition of total and unbound prednisolone in renal transplant patients receiving anticonvulsants

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Disposition of total and unbound prednisolone in renal transplant patients receiving anticonvulsants. Kidney transplant patients receiving phenytoin or phenobarbital may have decreased graft survival. These drugs have been shown to enhance the metabolism of glucocorticoids. We determined the disposition of total and unbound prednisolone in six stable kidney transplant patients receiving prednisone for immunosuppression and phenytoin or phenobarbital for a seizure disorder. Six similar patients not on anticonvulsants served as controls. A single intravenous dose of prednisolone was administered, and plasma samples were analyzed for prednisolone using a high-performance liquid chromatographic assay. Equilibrium dialysis was used to determine unbound prednisolone concentrations. Pharmacokinetic analysis showed that the half-life of prednisolone was shorter in the anticonvulsant group compared to the controls, based on both total (2.3 ± 0.4 vs. 3.4 ± 0.2 hr (SD), $P < 0.01$) and unbound (1.7 ± 0.3 vs. 2.4 ± 0.2 hr, $P < 0.01$) concentrations. Total drug clearance was 10.4 ± 2.8 liters/hr (0.171 ± 0.087 liters/hr · kg) in the anticonvulsant group versus 7.2 ± 1.2 liters/hr (0.100 ± 0.014 liters/hr · kg) in the controls ($P < 0.05$). Unbound prednisolone clearance was 57.2 ± 12.1 versus 46.4 ± 8.7 liters/hr ($P > 0.05$) and for weight-corrected estimates 0.886 ± 0.224 liters/hr · kg versus 0.644 ± 0.115 liters/hr · kg ($P < 0.05$) in the two groups, respectively. Thus, the disposition of prednisolone is altered by anticonvulsants in kidney transplant patients and may require dose alteration.

Disparition de la prednisolone totale et libre chez des transplantés rénaux recevant des anticonvulsivants. Les transplantés rénaux recevant de la phénytoïne ou du phénobarbital pourraient avoir une survie du greffon diminuée. Ces médicaments se sont avérés capables de stimuler le métabolisme des glucocorticoïdes. Nous avons déterminé l'élimination de la prednisolone totale et libre chez six transplantés rénaux stables recevant de la prédnisone pour leur immunosuppression et de la phénytoïne ou du phénobarbital pour une épilepsie. Six malades identiques sans anticonvulsivants ont servi de contrôle. Une dose unique intraveineuse de prédnisolone a été administrée, et des échantillons plasmatiques ont été analysés pour la prédnisolone en utilisant un dosage par chromatographie liquide à haute pression. Une dialyse à l'équilibre a été utilisée pour déterminer les concentrations de prédnisolone non liée. L'analyse pharmacocinétique a montré que la demi-vie de la prédnisolone était plus courte dans le groupe aux anticonvulsivants par rapport aux contrôles, qu'il s'agisse des concentrations totales (2,3 ± 0,4 contre 3,4 ± 0,2 hr (SD), $P < 0,01$) ou libres (1,7 ± 0,3 contre 2,4 ± 0,2 hr, $P < 0,01$). La clearance totale du médicament était de 10,4 ± 2,8 litres/hr (0,171 ± 0,087 litres/hr · kg) dans le groupe anticonvulsivant contre 7,2 ± 1,2 litres/hr (0,100 ± 0,014 litres/hr · kg) chez les contrôles ($P < 0,05$). La clearance de la prédnisolone non liée était de 57,2 ± 12,1 contre 46,4 ± 8,7 litres/hr ($P > 0,05$), et après correction pour le poids de 0,886 ± 0,224 litres/hr · kg contre 0,644 ± 0,115 litres/hr · kg ($P < 0,05$) dans les deux groupes, respectivement. Ainsi, l'élimination de la prédnisolone est altérée par les anticonvulsivants chez les transplantés rénaux et peut imposer des modifications de la dose.

Retrospective studies [1, 2] and case reports [3] have indicated that renal graft survival is decreased in kidney transplant patients receiving anticonvulsant medications such as phenytoin and phenobarbital. Other studies [4–12] in healthy volunteers and patients (with various disease states) have shown that the plasma clearance of various glucocorticoids (dexamethasone, hydrocortisone, methylprednisolone, and prednisolone) is increased in the presence of these anticonvulsants, presumably due to stimulation of hepatic drug metabolizing enzymes. Thus, decreased graft survival in transplant patients receiving anticonvulsants may be related to increased metabolism of prednisolone. Previous studies of glucocorticoid metabolism [4–12] were based only on measurement of total (bound and unbound) glucocorticoid concentrations. Determination of unbound prednisolone concentrations is important since prednisolone exhibits concentration-dependent pharmacokinetics [13–16], and it is the unbound drug which is believed to be pharmacologically active [17, 18].

The objective of this study was to compare the pharmacokinetic disposition of total and unbound prednisolone (the active metabolite of prednisone) in kidney transplant patients receiving anticonvulsants with a control group of patients. We found that anticonvulsants increased the clearance of both total and unbound prednisolone.

Methods

Study design. The present study included six clinically stable patients who had received a kidney transplant and were taking anticonvulsants and six similarly matched control transplant patients. The study was approved by the Committee on Human Research of the University of California at San Francisco. Individual patient characteristics are shown in Table 1 and anticonvulsant dosage regimens are shown in Table 2. All patients on anticonvulsants had been taking these drugs regularly for 5 weeks to 6 years. One patient (A4, C4) participated in both control and treatment groups, once prior to phenytoin

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Table 1. Patient characteristics

Patient no.	Age years	Sex	Weight kg	Serum creatinine mg/dl	Time since transplant months	C/NC[a]	Daily prednisone dose/duration mg/months	Peak hydrocortisone concentration[b] ng/ml
Anticonvulsant group								
A1	22	M	51	1.0	25	NC	15/25	144
A2	32	M	64	1.8	67	NC	15/52	79
A3	18	M	85	2.6	12	C	35/6	91
A4	27	M	84	1.1	39	NC	30/0.5	27
A5	38	M	70	1.6	23	NC	15/3	14
A6	16	M	45	1.1	3	NC	30/1.5	44
Mean	26		66	1.5	28			67
SD	8		16	0.6	23			48
Control group								
C1	56	M	78	1.6	18	NC	15/5	35
C2	30	F	82	2.6	23	C	20/8	<10
C3	28	M	60	1.1	13	NC	12.5/0.5	<10
C4	27	M	81	1.1	38	NC	15/1	17
C5	43	M	62	1.6	2	NC	30/0.5	114
C6	35	M	70	2.0	10	NC	20/4	<10
Mean	37		72	1.7	17			33
SD	11		10	0.6	12			41

[a] C/NC represents cushingoid/noncushingoid.
[b] The values represent the pre-study dose concentrations.

Table 2. Anticonvulsant dosage regimen

Patient no.	Anticonvulsant	Dose mg/day	Plasma concentration mg/liter
A1	Phenytoin	400	18
A2	Phenytoin	300	7
A3	Phenytoin	400	8
	Phenobarbital	90	17
A4	Phenytoin	300	12
A5	Phenobarbital	60	6
A6	Phenobarbital	60	13

treatment and again 5 weeks after its initiation. All patients had satisfactory kidney function and tests of liver function and serum albumin levels were in the normal range for both groups. In addition to their single daily maintenance dose of prednisone, patients were receiving azathioprine and various other drugs. However, no patients were taking any other medication known to alter drug metabolism.

Each patient received a single intravenous bolus dose of prednisolone (similar to their usual oral prednisone dose, or 0.2 mg/kg) as prednisolone sodium phosphate, Hydeltrasol® (Merck, Sharp & Dohme, West Point, Pennsylvania; see Table 3 for doses). Venous blood samples, 10 ml each, were obtained before the intravenous dose and at 0.04, 0.08, 0.17, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hr after the dose. Blood samples were collected in heparinized Vacutainer® tubes (stoppers removed) and immediately centrifuged; the plasma was harvested and then frozen at −70°C until assayed.

Assay and protein binding. Prednisolone and hydrocortisone concentrations in plasma were determined using a specific high-performance liquid chromatographic (HPLC) assay [19]. This assay allows the simultaneous measurement of these glucocorticoid compounds. The lower limit for routine analysis was 10 ng/ml. The intraday variability was 1 to 3% and the interday variability was 3 to 10% (coefficient of variation).

The plasma protein binding of prednisolone was determined in all plasma samples by equilibrium dialysis [20]. Briefly, 0.5 ml of plasma was equilibrated against 0.5 ml of isotonic Krebs-Ringer buffer (pH 7.4) containing $[6,7(n)\text{-}^3H]$ prednisolone at 37°C for 16 hr. Calculations of the bound and unbound prednisolone concentrations postdialysis were made correcting for the redistribution of drug and volume changes occurring during the dialysis procedure [21].

Data analysis. Protein binding. The binding of prednisolone to plasma proteins is concentration-dependent [14, 20–22] and may be described by the following function:

$$P'_{bnd} = \frac{CAP_1\, P'_u}{KD_1 + P'_u} + S\, P'_u \qquad (1)$$

where P'_{bnd} is the concentration of bound prednisolone and P'_u is the unbound concentration after dialysis; CAP_1 and KD_1 are the binding capacity and dissociation constant of a saturable binding site (transcortin), and S is the ratio of capacity to dissociation constant (CAP_2/KD_2) of a nonsaturated binding site (albumin). After estimates of the binding parameters CAP_1, KD_1, and S for prednisolone were obtained from each study patient, the unbound concentration was derived from the equation:

$$P_u = \frac{B + \sqrt{B^2 + 4\, A\, KD_1\, P_{tot}}}{2A} \qquad (2)$$

where P_u is the estimate of the unbound concentration of prednisolone in vivo, $B = (P_{tot} - CAP_1 - KD_1\, A)$, $A = 1 + S$, and P_{tot} is the total concentration of prednisolone measured in the patient's plasma by HPLC prior to equilibrium dialysis. Protein binding parameters were estimated using unweighted nonlinear least squares regression [23].

Pharmacokinetics. A two-compartment model with bolus input was used to describe plasma prednisolone concentrations of total and unbound drug. The pharmacokinetic parameters of prednisolone were estimated using nonlinear least squares regression [24]. Observed concentrations were weighted by the

Table 3. Pharmacokinetic parameters of total prednisolone

Patient no.	Prednis- olone dose *mg*	$T_{1/2}$ *hr*	CL *liters/hr*	CL *liters/ hr · kg*	V_{ss} *liters*	V_{ss} *liters/kg*
Anticonvulsant group						
A1	12.3	1.9	9.7	0.191	23.2	0.46
A2	12.5	2.1	8.4	0.132	23.3	0.37
A3	17.0	2.6	11.9	0.140	37.2	0.44
A4	16.4	2.6	7.8	0.093	28.1	0.34
A5	15.0	2.8	9.4	0.133	36.0	0.51
A6	30.0	1.8	15.3	0.337	33.2	0.73
Mean	17.2	2.3	10.4	0.171	30.2	0.47
SD	6.6	0.4	2.8	0.087	6.2	0.14
Control group						
C1	15.0	3.1	9.0	0.116	39.4	0.51
C2	17.5	3.6	8.3	0.100	40.3	0.49
C3	15.0	3.5	6.7	0.111	32.2	0.53
C4	16.0	3.2	6.1	0.076	27.6	0.34
C5	15.0	3.2	6.2	0.101	23.8	0.38
C6	15.0	3.6	6.8	0.096	34.9	0.50
Mean	15.6	3.4	7.2	0.100	33.0	0.46
SD	1.0	0.2	1.2	0.014	6.6	0.08
P { *t* test		0.0003	0.026	0.077	0.452	0.828
{ Mann-Whitney		< 0.01	< 0.05	< 0.05	> 0.05	> 0.05

Table 4. Prednisolone binding parameters[a]

Patient group	CAP_1 *ng/ml*	KD_1 *ng/ml*	S
Anticonvulsant	210 ± 53.6	18.7 ± 8.1	1.4 ± 0.5
Control	187.5 ± 113.6	17.3 ± 8.3	2.0 ± 0.9
P { *t* test	0.66	0.78	0.14
{ Mann-Whitney	> 0.05	> 0.05	> 0.05

[a] Values are mean ± SD.

reciprocal of the value squared. Half-life was calculated by dividing 0.693 by the terminal elimination rate constant (λz) estimated from the pharmacokinetic model. The area under the plasma concentration-time curve (AUC) was obtained using the log-trapezoidal rule and the remaining AUC beyond the last measured data point was estimated by dividing the predicted value for the last data point by λz [24]. Estimates of plasma clearance (CL) and volume of distribution at steady state (V_{ss}) were calculated using the noncompartmental equations:

$$CL = \frac{Dose}{AUC} \qquad (3)$$

and

$$V_{ss} = Dose\ (AUMC)/(AUC)^2 \qquad (4)$$

where AUMC is the area under the first moment of the plasma prednisolone concentration-time curve, using the log-trapezoidal rule [25, 26].

Statistical analysis. Statistical comparisons between the two patient groups were made using the *t* test on unpaired data and the Mann-Whitney Rank Sign Test on the PROPHET computer system [27]. Both tests were applied to all comparisons and a statistically significant difference was defined as $P < 0.05$. If disagreement was observed, the results of the nonparametric test were used to determine significance. Results are reported as mean ± SD.

Results

The pharmacokinetic parameters based on total prednisolone concentrations are listed in Table 3. A significantly shorter mean prednisolone half-life of 2.3 ± 0.4 hr was observed in the

anticonvulsant group, compared to 3.4 ± 0.2 hr in the control patients. This was accounted for by a significant increase in total plasma drug clearance, 10.4 ± 2.8 liters/hr (0.171 ± 0.087 liters/hr · kg) versus 7.2 ± 1.2 liters/hr (0.100 ± 0.014 liters/hr · kg) in the two respective groups. No differences were noted in mean steady-state volume of distribution between the two groups. Figure 1 illustrates the plasma prednisolone concentration-time profile for total drug in the one patient (A4, C4) who was studied in the presence and absence of phenytoin.

In Table 4 are summarized the protein binding parameters of prednisolone. No significant differences were observed in values for CAP_1, KD_1, and S of prednisolone in the two groups of patients.

Table 5 lists the pharmacokinetic parameters based on unbound prednisolone concentration. A significant decrease in half-life for the anticonvulsant group was found, being 1.7 ± 0.3 hr versus 2.4 ± 0.2 hr in the control patient group. For the anticonvulsant group unbound prednisolone clearance was higher, 57.2 ± 12.1 liters/hr versus 46.4 ± 8.7 liters/hr ($P > 0.05$); and for weight corrected values, 0.886 ± 0.224 liters/hr · kg versus 0.644 ± 0.115 liters/hr · kg ($P < 0.05$). Steady-state volume of distribution was lower, 101.1 ± 33.2 liters (1.53 ± 0.39 liters/kg) versus 145.7 ± 40.9 liters (2.03 ± 0.67 liters/kg) compared to the controls. However, these differences were not statistically different. Figure 2 illustrates the plasma prednisolone concentration-time profile for unbound drug in the same patient as is shown in Figure 1. Note the greater differences in drug concentrations observed between the two study conditions compared to that using total prednisolone concentrations.

Patients receiving anticonvulsants exhibited higher mean morning (pre-dose) hydrocortisone peak concentrations of 67 ± 48 ng/ml than did control patients with 33 ± 41 ng/ml (Table 1). Analysis of all plasma samples obtained during the pharmacokinetic study for hydrocortisone concentration above the detection limit of 10 ng/ml revealed that hydrocortisone was present almost twice as often in the anticonvulsant group, 22% (16 out of 72 samples), compared to controls, 13% (10 out of 77 samples).

Discussion

In 1977, Wassner et al [2] reported results of a retrospective study showing that the 1- and 2-yr graft survival for 25 grafts in patients on anticonvulsant medication was 32 and 6%, respectively, compared to 76 and 67% in 127 control grafts. Phenobarbital was the primary anticonvulsant used, although some patients required phenytoin or ethosuximide.

Our clinical observations, as well as that of other investigators [3], agree with the findings of Wassner et al [2], that good allograft function can be more difficult to maintain in transplant
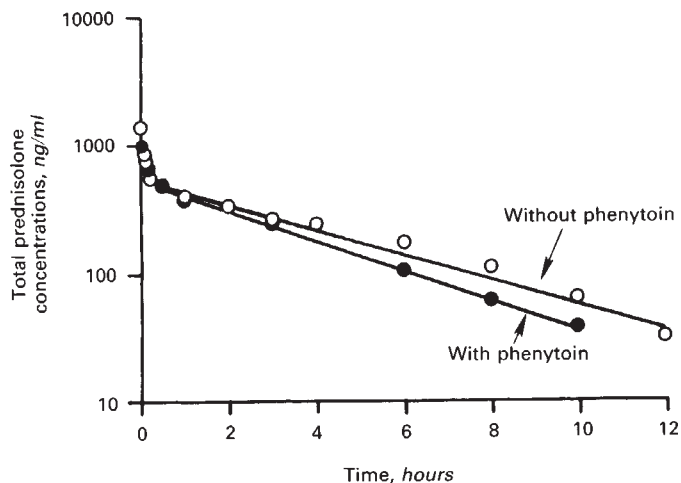
Fig. 1. *The plasma total prednisolone concentration-time profile in patient A4,C4 studied in the presence (●) and the absence (○) of phenytoin.*
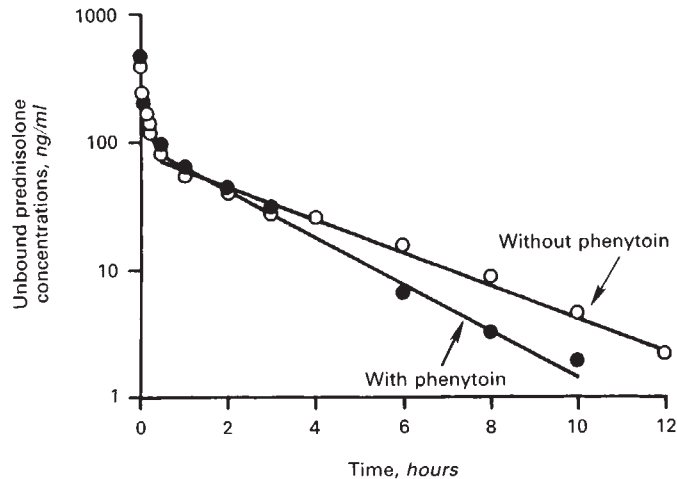

Fig. 2. *The plasma unbound prednisolone concentration-time profile in patient A4,C4 studied in the presence (●) and the absence (○) of phenytoin.*

Table 5. Pharmacokinetic parameters of unbound prednisolone

Patient no.	$T_{1/2}$ hr	CL liters/hr	CL liters/ hr · kg	V_{ss} liters	V_{ss} liters/kg
Anticonvulsant group					
A1	1.5	66.9	1.315	93.1	1.83
A2	1.6	44.9	0.706	73.9	1.16
A3	1.8	71.8	0.845	107.6	1.27
A4	1.7	59.8	0.715	121.6	1.45
A5	2.1	58.4	0.833	151.0	2.15
A6	1.4	41.1	0.904	59.3	1.31
Mean	1.7	57.2	0.886	101.1	1.53
SD	0.3	12.1	0.224	33.2	0.39
Control group					
C1	2.4	50.6	0.649	158.8	2.04
C2	2.4	54.3	0.660	156.8	1.91
C3	2.8	50.2	0.832	187.6	3.11
C4	2.4	47.5	0.587	138.4	1.71
C5	2.2	29.5	0.479	68.8	1.11
C6	2.5	46.2	0.656	164.0	2.33
Mean	2.4	46.4	0.644	145.7	2.03
SD	0.2	8.7	0.115	40.9	0.67
P { t test	0.0003	0.107	0.040	0.065	0.138
{ Mann-Whitney	< 0.01	> 0.05	< 0.01	> 0.05	> 0.05

patients on anticonvulsants and greater doses of prednisone may be required. Wassner et al [2] noted that in two patients in whom anticonvulsant medication could not be discontinued, the daily doses of prednisone needed were greater than 1 mg/kg. Several studies in healthy volunteers and patients having a variety of disease states have reported the effect of phenytoin or phenobarbital on increasing the metabolism of glucocorticoids and/or decreasing its clinical effects [4–12]. Sells et al [5] found the half-life of methylprednisolone significantly shorter in kidney transplant patients receiving phenobarbital or phenytoin for a seizure disorder with or without a functioning allograft, compared to controls. Petereit and Meikle [4] studied the effect of 3 weeks of phenytoin administration on prednisolone pharmacokinetics in five healthy subjects. The clearance of prednis-

olone increased by 77 ± 46% of the baseline value of 1.16 ± 0.29 ml/min/kg, and half-life decreased by 45 ± 9.2% of the control value of 3.2 ± 0.4 hr. Distribution volume and bioavailability of prednisolone were not significantly altered by the phenytoin.

Unlike previous studies [4–12], we were able to measure the concentration of unbound prednisolone in plasma as well as total drug concentrations. Because the protein binding of prednisolone changes with concentration, clearance estimates based on total drug concentrations are misleading, since they are confounded by the variation in binding [14]. Both total and unbound prednisolone plasma half-life were significantly shorter in the anticonvulsant group. When based on total drug, this decrease in half-life was accounted for by a corresponding significant increase of approximately 44% in plasma clearance, from 7.2 ± 1.2 to 10.4 ± 2.8 liters/hr (71% increase for weight-corrected values; Table 3). No change in steady-state volume of distribution was observed. The difference in plasma clearance is similar to that found by Petereit and Meikle [4] in their healthy subjects. However, if the patient (A6) who received the largest prednisolone dose (who also had the largest total clearance) is excluded, then the increased clearance in the anticonvulsant group is only 31% (38% using weight-corrected estimates). The higher total clearance due to the higher dose in this patient is consistent with the concentration-dependent pharmacokinetic disposition of this drug [14–16].

With regard to unbound prednisolone concentrations, drug clearance, and distribution volume, differences in these parameters between the two groups are in a direction consistent with the significant change in half-life, that is, clearance increases, and volume decreases in the anticonvulsant group. Unbound prednisolone clearance was 46.4 ± 8.7 liters/hr in the control patients and 57.2 ± 12.1 liters/hr in those on anticonvulsants, a difference of 23% (Table 4). The weight-corrected estimates of unbound clearance exhibited a significant difference of 38% between the two groups. This is approximately half that observed for differences in estimates of clearance based on total drug concentrations. Reasons for a lower mean steady-state volume of distribution in the anticonvulsant group compared to

the controls are unknown. No significant differences were observed in the in vivo plasma protein binding parameters of prednisolone in the two groups. At the receptor level, Ballard et al [28] have shown that phenobarbital and phenytoin inhibit binding of ^{3}H-dexamethasone by glucocorticoid receptors of rat hepatoma (HTC) cells only when they are at toxic-to-lethal concentrations. For the patients in our study, plasma concentrations of phenytoin and phenobarbital were either within or slightly below the therapeutic range (Table 2).

Petereit and Meikle [4] observed that in healthy subjects the average oral dose of prednisolone needed to suppress the 8 A.M. plasma hydrocortisone level to 50 ng/ml was approximately doubled after phenytoin. It is interesting to note that our patients on anticonvulsants had over a twofold greater mean morning peak hydrocortisone concentration. Measurable hydrocortisone levels were also found almost twice as often in plasma samples obtained during the study, compared to the control group. This apparently reflects less suppression of the hypothalamic-pituitary-adrenal axis and a more normal circadian rhythm of endogenous steroid production.

In conclusion, the disposition of prednisolone is altered in kidney transplant patients receiving phenobarbital or phenytoin. A significantly shorter half-life is observed for total and unbound prednisolone. However, the increase in plasma clearance based on unbound prednisolone is 23 to 38%, which is less than that observed for total drug concentrations, 44 to 71%. Thus, the reported differences in therapeutic response in patients taking anticonvulsants may not be explained entirely by increased unbound corticosteroid clearance, and additional factors are involved in determining the appropriate steroid dose in these transplant patients.

Acknowledgments

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