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Nonlinear Plasma Protein Binding and Haemodialysis Clearance of Prednisolone*

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Summary. The impact of nonlinear plasma protein binding of a drug on its removal by haemodialysis has been quantified. Prednisolone 10–100 mg was given i.v. to 10 renal transplant patients on haemodialysis for acute tubular necrosis. Dialysate and afferent and efferent blood samples were collected simultaneously in 67 instances. Total and unbound prednisolone in plasma and its total concentration in blood and dialysate were assessed by high performance liquid chromatography and equilibrium dialysis. The amount of prednisolone lost, as measured directly in the dialysate (21.8 ± 4.4 μg/min, $\bar{x}$ ± SE), was predictable from the afferent-efferent blood concentration differences (20.1 ± 4.8 μg/min), but not from measurements of total afferent-efferent prednisolone concentrations in plasma (13.1 ± 3.0 μg/min). The amount of prednisolone lost in the dialysate increased linearly with unbound (r^2 = 0.96) and hyperbolically with the total prednisolone concentration in plasma. The latter hyperbolic relationship is adequately described by the equation for nonlinear plasma protein binding, using the affinity and capacity constants of albumin and transcortin for prednisolone (r^2 = 0.98). Thus, the haemodialysis clearance of total prednisolone is concentration-dependent, while the clearance of unbound prednisolone is constant (76 ml/min). Free clearance values or measurements of afferent-efferent blood concentrations are mandatory for a drug showing nonlinear plasma protein binding in order to predict the amount lost in the dialysate.

Key words: haemodialysis, protein binding, prednisolone; clearance, renal transplant, free clearance, dialysate loss

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Renal transplant patients often undergo haemodialysis for acute tubular necrosis following transplantation. The purpose of the present study was to determine the effect of haemodialysis on the plasma level of prednisolone, which has not been previously detailed [1, 2]. Prednisolone exhibits nonlinear plasma protein binding [3, 4, 5], and the influence of nonlinear plasma protein binding of an agent on its removal by haemodialysis and the appropriate technique to assess this behaviour have not previously been described. This is of interest because an increasing number of drugs and hormones have concentration-dependent plasma protein binding in the therapeutic range [6–12].

It is reasonable to assume that assessment of changes in the half-life of a drug with nonlinear plasma protein binding is of little value in providing a guide to dosage adjustment, since determination of half-life is dependent both on clearance and volume of distribution. The latter parameters are not constants, but are a function of the plasma concentration for agents exhibiting nonlinear plasma protein binding [4, 5]. Haemodialysis clearance values calculated from dialysate flux and total plasma concentrations [13] are considered useful constants in predicting drug loss into the dialysate. However, these constants may become a complicated function of the binding parameters relating free to total plasma concentration, if the free concentration of a drug in plasma changes as a function of the total concentration, since only the free drug is expected to diffuse from plasma water into the dialysate. Clearance values calculated from dialysate flux and unbound plasma concentrations on the other hand may be concentration-independent constants, and may be useful, therefore, in predicting drug loss into the dialysate. If, for technical reasons, drug cannot be measured in dialysate, the amount removed of a drug with nonlinear plasma protein binding must be calculated on

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Table 1. Prednisolone dose administered, mean rate of loss of prednisolone during haemodialysis, mean free fraction and volume of distribution of prednisolone

Patient	Prednisolone dose		Mean loss of prednisolone in haemodialysis				Mean free fraction	Volume of distribution
			Percentage of dose/h	Amount/min [μg/min]				
				total	by diffusion	by ultrafiltration		
	[mg]	[mg/kg]		$\overline{Z}_{Dtot}$	$\overline{Z}_{Ddi}$	$\overline{Z}_{Du}$		[l/kg]
I	50	0.67	2.3	19.2	17.5	1.7	0.367	0.59
II	50	0.47	2.3	19.3	17.5	1.8	0.397	0.46
III	120	2.10	2.6	51.2	49.3	1.9	0.476	1.15
IV	100	1.15	2.1	35.1	31.9	3.2	0.496	0.85
V	50	0.75	3.1	26.1	23.3	2.8	0.348	0.78
VI	40	0.56	1.8	12.0	10.5	1.5	0.300	0.60
VII	80	0.98	2.3	30.0	26.9	3.1	0.344	0.89
VIII	80	1.12	2.7	36.4	30.8	5.6	0.391	1.08
IX	10	0.23	3.5	5.9	5.6	0.3	0.320	0.55
X	20	0.25	1.4	4.5	4.3	0.2	0.338	0.75

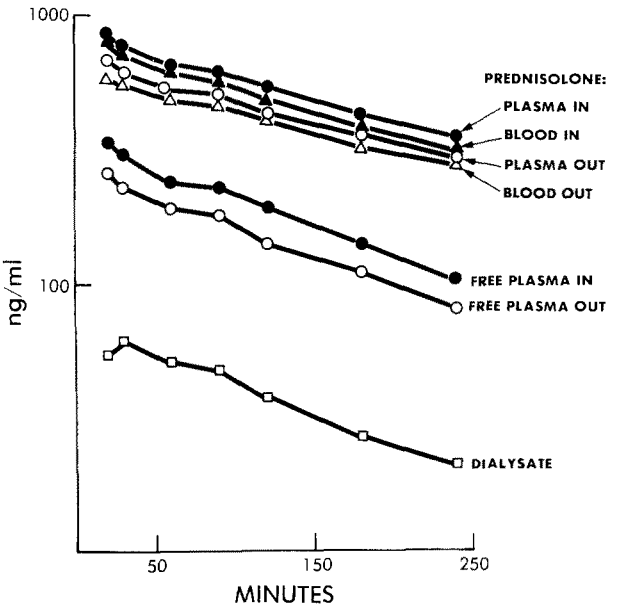

Fig. 1. Semilogarithmic plot of the following different concentrations of prednisolone [ng/ml] versus time over the dialysis period for Patient I: plasma in = afferent plasma concentration (C_{Pa}), plasma out = efferent plasma concentration (C_{Pe}); blood in = afferent blood concentration (C_{Ba}), blood out = efferent blood concentration (C_{Be}); free plasma in = free concentration in afferent plasma (C_{PaFree}), free plasma out = free concentration in efferent plasma (C_{PeFree}) and dialysate = dialysate outlet concentration

the basis of blood flow and afferent-efferent concentration differences, using whole blood concentrations rather than plasma concentrations. This is because the concentration-dependent changes in distribution of the drug may not permit accurate mass balance consideration on the basis solely of plasma levels.

In an attempt to substantiate these theoretical predictions, and to provide guidelines for future kinetic studies of endogenous and exogenous agents exhibiting nonlinear plasma protein binding in patients on haemodialysis, the kinetics of prednisolone have been investigated. Total and unbound haemodialysis clearance of prednisolone were determined and the amount of prednisolone lost in the dialysate was calculated, as directly assessed in the dialysate and from plasma and blood concentration measurements in afferent and efferent blood, using a high performance liquid chromatography and recently developed equilibrium dialysis method [14, 15].

Material and Methods

The present study comprised 10 renal transplant patients on haemodialysis for acute tubular necrosis. The dialyzer used was the C-DAK, 1.8 m^2 (Cordis Dow Corp., Miami, Fla., USA), the delivery system was the Centry II (Cobe Laboratories Inc., Lakewood, Colo., USA). The amount of prednisolone administered to the patients is given in Table 1. The total amount of exogenous glucocorticoids administered on the study day was identical with that prescribed by the physician treating the patient; only the route of administration was changed, i.e. intravenous instead of the usual oral dosing was instituted. Informed consent was obtained from all patients according to a protocol approved by the Committee on Human Research of the University of California, San Francisco, USA.

On the study day the dose of prednisolone was administered 20 min prior to beginning dialysis treatment. Before prednisolone was injected, a venous

blood sample was collected. Simultaneous samples of dialysate, afferent and efferent blood were collected 10, 20, 30, 60, 90 and 180 min after dialysis was started, and immediately before it was discontinued (Fig. 1).

The haematocrit of all afferent and efferent blood samples was determined twice and the arithmetic mean used in further calculations. The total concentration of prednisolone in whole afferent and efferent blood and plasma, as well as in the dialysate, was determined by high performance liquid chromatography [14]. Equilibrium dialysis of all plasma samples was carried out to quantify the concentration of unbound prednisolone as previously described [15, 16].

Data Analysis

Estimates of the loss of prednisolone were obtained 1) by directly measuring prednisolone in the dialysate and 2) by calculating differences in the afferent-efferent concentrations in blood or plasma:

1) The total loss of prednisolone at each sampling time point (Z_{Dtot}), as directly measured in the dialysate, was calculated as:

$$Z_{Dtot} = Q_D \cdot C_D, \tag{1}$$

where Q_D is the flow of dialysate, and C_D the prednisolone concentration in the dialysate. The value of Q_D was obtained by directly measuring the dialysate flow. In the „Results" section, the loss of prednisolone is given in "µg", while plasma concentrations are given in "ng/ml". The mean total loss of prednisolone calculated for the entire haemodialysis period ($\bar{Z}_{Dtot}$) was obtained from the following equation:

$$\bar{Z}_{Dtot} = \frac{1}{t_2 - t_1} \int_{t_1}^{t_2} Q_D \cdot C_D \cdot dt, \tag{2}$$

where t_1 is the first sampling time point after commencement of haemodialysis, and t_2 is the last sampling time point before haemodialysis was discontinued. The integration was performed by the linear trapezoidal rule [17]. For each sampling time point, the volume of ultrafiltration (Q_F) was calculated as:

$$Q_F = Q_B \left(\frac{H_e}{H_a} - 1\right), \tag{3}$$

where Q_B is the efferent blood flow and H_a and H_e are the haematocrit values in afferent and efferent blood, respectively. Equation 3 is derivable from mass balance considerations of the red blood cells crossing the haemodialysis machine. Assuming that only free prednisolone from the afferent plasma (C_{PaFree}) is ultrafiltrable, the amount of prednisolone, lost by ultrafiltration (Z_{Du}) is obtained as follows:

$$Z_{Du} = C_{PaFree} \cdot Q_B \left(\frac{H_e}{H_a} - 1\right). \tag{4}$$

The mean amount of prednisolone lost by ultrafiltration ($\bar{Z}_{Du}$) in the course of the entire haemodialysis period was calculated according to the following equation:

$$\bar{Z}_{Du} = \frac{1}{t_2 - t_1} \int_{t_1}^{t_2} C_{PaFree} \cdot Q_B \left(\frac{H_e}{H_a} - 1\right) dt. \tag{5}$$

Subtracting from the total loss of prednisolone in the dialysate (Equ. 2) the amount lost by ultrafiltration (Equ. 5) gives the mean amount of prednisolone lost in the dialysate by diffusion ($\bar{Z}_{Ddi}$).

Dialysis clearance values (Cl) were obtained by dividing the amount of prednisolone lost in the dialysate by diffusion by the total afferent concentration of prednisolone in blood (Cl_B) or plasma (Cl_P), or by the free prednisolone concentration in plasma (Cl_{Free}).

2) The loss of prednisolone (c) at each sampling time point was calculated from the afferent-efferent blood concentrations (Z_{cB}) using the following equation:

$$Z_{cB} = Q_B (C_{Ba} - C_{Be}). \tag{6}$$

The corresponding mean value for the entire haemodialysis period ($\bar{Z}_{cB}$) is:

$$\bar{Z}_{cB} = Q_B \frac{1}{t_2 - t_1} \left(\int_{t_1}^{t_2} C_{Ba} \cdot dt - \int_{t_1}^{t_2} C_{Be} \cdot dt \right). \tag{7}$$

Analogous calculations (Z_{cP}, $\bar{Z}_{cP}$) were performed using the afferent-efferent plasma concentrations of prednisolone:

$$Z_{cP} = Q_B (1 - H_e) (C_{Pa} - C_{Pe}) \tag{8}$$

$$\bar{Z}_{cP} = Q_B \frac{1}{t_2 - t_1} (1 - H_e) \left(\int_{t_1}^{t_2} C_{Pa} \cdot dt - \int_{t_1}^{t_2} C_{Pe} \cdot dt \right). \tag{9}$$

From the plasma concentration of prednisolone and its coefficient of partition (K) between red blood cells and plasma (Lee et al. [18]), the loss of predniso-

lone at each sampling time point (Z_{cPK}) and in the entire haemodialysis treatment period ($\bar{Z}_{cPK}$) were calculated:

$$Z_{cPK} = Q_B (1 - H_e + KH_e)(C_{Pa} - C_{Pe}) \quad (10)$$

$$\bar{Z}_{cPK} = Q_B \frac{1}{t_2 - t_1} (1 - H_e + KH_e) \left(\int_{t_1}^{t_2} C_{Pa} \cdot dt - \int_{t_1}^{t_2} C_{Pe} \cdot dt \right). \quad (11)$$

K was obtained by dividing the concentration of prednisolone in red blood cells by its concentration in the corresponding plasma.

The nonlinearity between the amount of prednisolone lost by diffusion, as measured directly in the dialysate (Z_{Ddi}), and the total concentration of prednisolone in the afferent plasma (C_{Pa}; Equation 14; Fig. 6) was fitted by means of the equation derived for nonlinear protein binding of prednisolone. The nonlinear protein binding of prednisolone may be expressed by the following equation [5, 22]:

$$b = \frac{CAP_T \cdot C_{PaFree}}{KD_T + C_{PaFree}} + \frac{CAP_A \cdot C_{PaFree}}{KD_A}, \quad (12)$$

where b is the concentration of prednisolone bound to transcortin (T) and albumin (A); CAP_T, KD_T, CAP_A and KD_A are the binding capacities and dissociation constants of transcortin and albumin, respectively, and C_{PaFree} is the concentration of free prednisolone.

The free concentration was derived from Equ. (12) by substituting $C_{Pa} - C_{PaFree}$ for b and solving for C_{PaFree}; i.e.

$$C_{PaFree} = \frac{C_{Pa} - CAP_T - M \cdot KD_T + \sqrt{(C_{Pa} - CAP_T - M \cdot KD_T)^2 + 4M \cdot KD_T \cdot C_{Pa}}}{2\,M}, \quad (13)$$

where $M = 1 + (CAP_A/KD_A)$.

The diffusional loss of prednisolone in the dialysate (Z_{Ddi}) as a function of the total prednisolone concentration and of the affinity and capacity constants is:

$$Z_{Ddi} = Cl_{Free} \frac{C_{Pa} - CAP_T - M \cdot KD_T + \sqrt{(C_{Pa} - CAP_T - M \cdot KD_T)^2 + 4M \cdot KD_T \cdot C_{Pa}}}{2\,M}. \quad (14)$$

The binding parameters for prednisolone CAP_T, KD_T and CAP_A, KD_A were estimated for the entire population (Fig. 6) by nonlinear least squares regression with unit weighting ($r^2 = 0.98$) (Multifun [23]). The value of CAP_A was estimated using a molecular weight of 66 500 for albumin [24], and the mean albumin concentration (39,5 g/l) measured in plasma from the patient. The non-compartmental volume of distribution of prednisolone was calculated [19, 20]. The ratio of the area under the plasma concentration – time curve of unbound prednisolone to the area under the plasma concentration – time curve of total plasma prednisolone concentrations was used as a time-averaged measure of the fraction of plasma prednisolone not bound to plasma protein (mean free fraction).

Statistical analysis employed the *t*-test for paired observations [21].

Results

The mean total loss of prednisolone calculated for the entire dialysis period ($\bar{Z}_{Dtot}$) ranged from 4.5 µg/min to 51.2 µg/min (Table 1). The total loss of prednisolone rose with increase in the dose of prednisolone administered ($\bar{Z}_{Dtot}$ [µg/min] = 25.5 × dose [mg/kg] + 2.82; $r = 0.96$, $p < 0.001$; Fig. 2). During a 1 h period of haemodialysis, the total loss of prednisolone (± SD) was 2.4 ± 0.6% of the dose of prednisolone administered (Table 1). The percentage loss of prednisolone did not increase as a function of the dose of prednisolone administered ($r = 0.10$; $p > 0.05$; Fig. 3).

The predominant mode by which prednisolone was lost in the dialysate was diffusion (Table 1), i.e. 91.1 ± 3.7% ($\bar{x}$ ± SD) of the total loss. The loss of prednisolone by diffusion increased with the dose of prednisolone administered ($\bar{Z}_{Ddi}$ [µg/min] = 24.2 × dose [mg/kg] + 1.74; $r = 0.97$, $p < 0.001$; Table 1); the loss of prednisolone by diffusion, expressed as a percentage of the dose administered, did not increase as a function of the dose of prednisolone administered ($r = 0.09$, $p > 0.05$).

The mean free fraction of prednisolone in plasma calculated for the entire haemodialysis period ranged from 0.3 to 0.5 (Table 1). The free fraction increased as a function of the dose of prednisolone administered (free fraction = 0.086 × dose [mg/kg] + 0.306; $r = 0.74$, $p < 0.05$; Fig. 4). The volume of distribution of prednisolone was calculated both for total and unbound prednisolone. Only the volume of distribution of total prednisolone (Table 1) increased with the dose of prednisolone (volume of distribution [l/kg] = 0.343 × dose [mg/kg] + 0.486; $r = 0.83$; $p < 0.01$; Fig. 5; Table 1).

The measured amount of prednisolone lost by diffusion (Z_{Ddi}), expressed as a function of the unbound prednisolone concentration in the afferent plasma (C_{PaFree}), yielded a straight line (Fig. 6): Z_{Ddi}

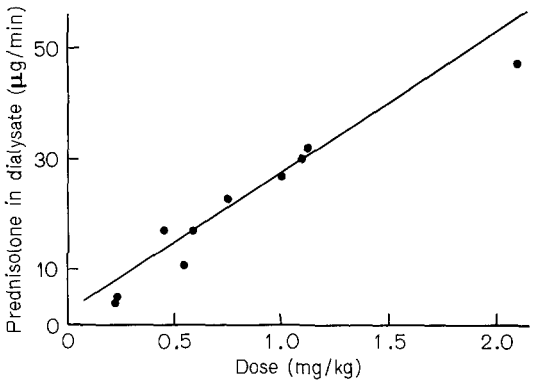

Fig. 2. For each patient the mean total loss of prednisolone ($\bar{Z}_{Dtot}$) calculated for the entire dialysis period (y-axis) is plotted against the administered dose of prednisolone (x-axis). The loss of prednisolone increases with increasing dose of prednisolone ($r = 0.96$, $p < 0.001$). The regression line is indicated by a solid line

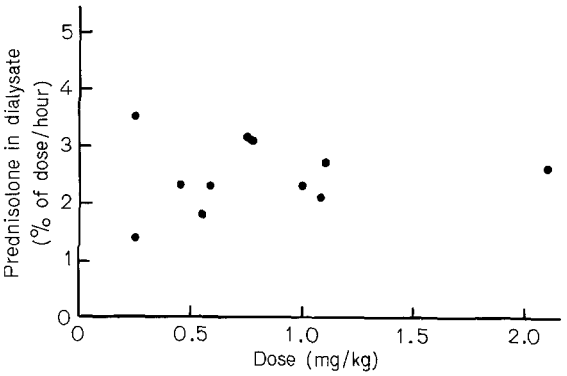

Fig. 3. Prednisolone loss in dialysate versus dose of prednisolone administered. The mean amount of prednisolone recovered in the dialysate in the course of a 1 h period of haemodialysis is expressed as a percentage of the dose of prednisolone administered (y-axis). The fraction of the dose lost in the dialysate is independent of the dose of prednisolone administered

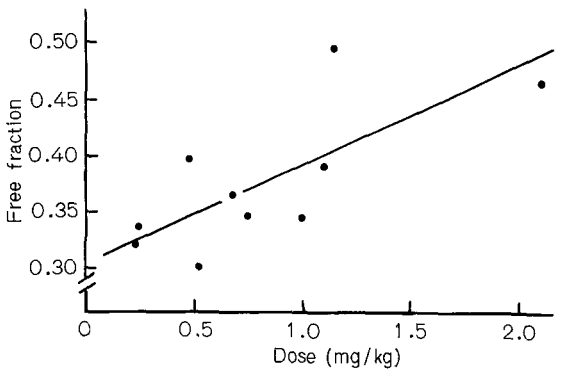

Fig. 4. Plot of mean free fraction of prednisolone versus the dose of prednisolone administered. The fraction of prednisolone not bound to plasma protein increases as a function of the dose of prednisolone administered ($r = 0.74$, $p < 0.05$)

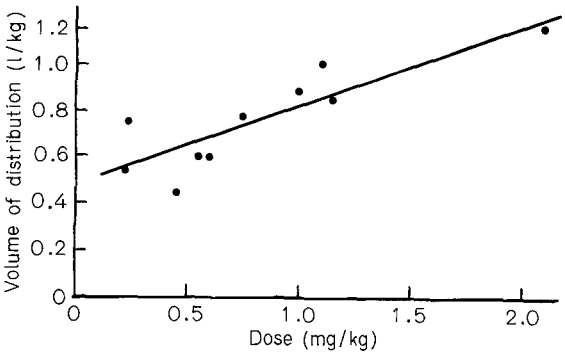

Fig. 5. Plot of volume of distribution of prednisolone versus dose of prednisolone administered: with increasing dose the volume of distribution increases ($r = 0.83$, $p < 0.01$)

[µg/min] = 0.076 × C_{PaFree} [ng/ml] − 0.031; r = 0.982; $p < 0.001$. The slope of the line, 76 ml/min, is the mean free clearance value for the entire population investigated. The unbound clearance (Cl_{Free}) did not change as a function of the total prednisolone concentration in plasma, as shown in the upper part of Fig. 7; in none of the 10 patients was there a significant increase or decrease in the unbound clearance as a function of the plasma prednisolone concentration. However, the clearance values obtained by assessing total plasma concentrations (Cl_P, lower part of Fig. 7) or blood concentrations (Cl_B, results not shown) of prednisolone increased significantly (p ranged from < 0.05 to < 0.001) with increasing plasma concentrations of prednisolone in 8 out of 10 patients. In the remaining 2 patients, i.e. Patients I and IX, a similar but non-significant tendency to increase was observed.

The nonlinearity between the amount of prednisolone lost by diffusion and the total concentration of prednisolone in plasma (Fig. 6) was fitted by means of the equation derived for nonlinear protein binding of prednisolone (Equ. 14). The following constants were obtained: capacity of transcortin (CAP_T) 38.1 µg%, capacity of albumin 20790 µg%, affinity of transcortin $\left(\frac{1}{KD_T}\right)$ 2.9 × 10^6 $\frac{L}{M}$, and affinity of albumin $\left(\frac{1}{KD_A}\right)$ 0.73 · 10^3 $\frac{L}{M}$.

Estimates of prednisolone loss in the dialysate were obtained by direct measurement of prednisolone in the dialysate, or by calculating the loss from the afferent-efferent plasma or blood concentration differences (Table 2). The results obtained by direct

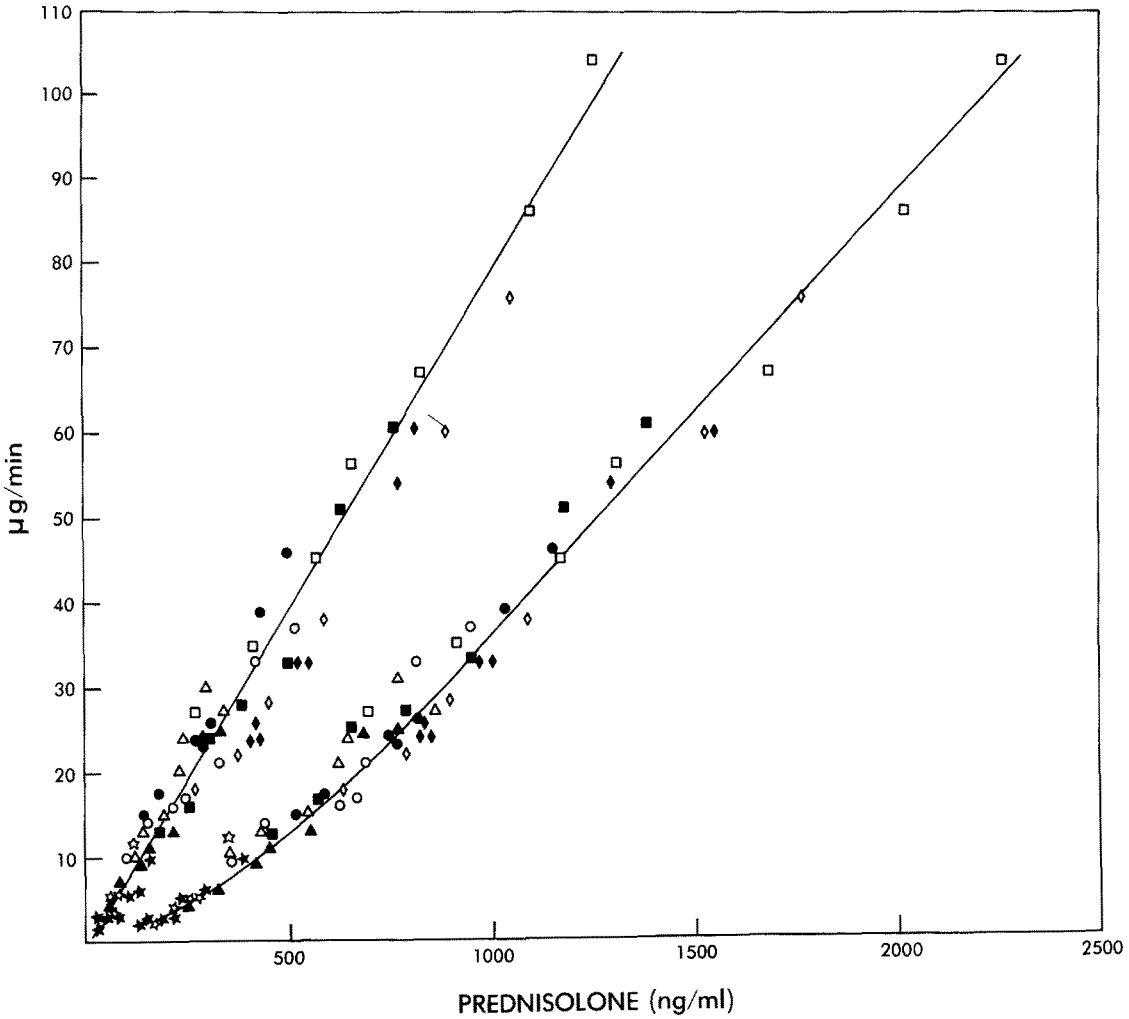

Fig. 6. Amount of prednisolone lost by diffusion (y-axis) versus the unbound (straight line) or total (curved line) concentration of prednisolone in plasma (x-axis). Patients: I ○, II △, III □, IV ◇, V ●, VI ▲, VII ■, VIII ◆, IX ☆, X ★

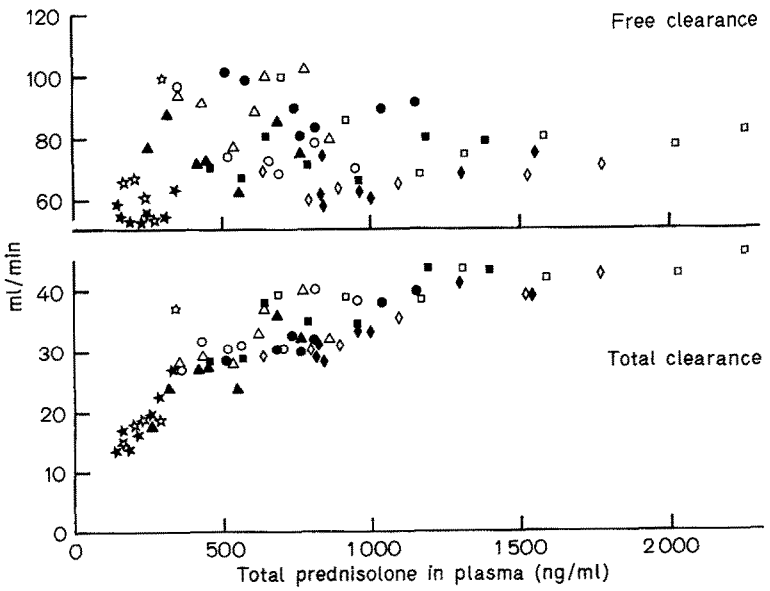

Fig. 7. On the x-axis the total concentration of prednisolone in the afferent plasma (C_{Pa}) is shown. On the y-axis of the upper and lower parts the free plasma clearance (Cl_{Free}) and total plasma clearance (Cl_P) are shown, respectively. With an increasing plasma concentration of prednisolone the total plasma clearance increases, whilst that of unbound prednisolone clearance do not. The same symbols are used as in Fig. 6

Table 2. Mean loss (± SE) of prednisolone [μg/min] by diffusion as measured directly in the dialysate ($\overline{Z}_{Ddi}$) and as calculated from the differences between afferent-efferent concentrations in plasma without ($\overline{Z}_{pP}$) and with ($\overline{Z}_{pPK}$) allowance for partitioning, or as calculated from afferent-efferent concentration differences in blood ($\overline{Z}_{cB}$)

Patient	Measured	Calculated by concentration differences		
		Plasma	Plasma corrected for partitioning	Blood
	$\overline{Z}_{Ddi}$	$\overline{Z}_{cP}$	$\overline{Z}_{cPK}$	$\overline{Z}_{cB}$
I	17.5	15.5	17.6	17.5
II	17.5	9.3	11.3	20.8
III	49.3	33.9	45.6	56.8
IV	31.9	20.8	25.4	27.1
V	23.3	9.7	11.1	15.2
VI	10.5	10.1	11.8	12.1
VII	26.9	12.4	16.8	17.5
VIII	30.8	14.9	17.7	26.7
IX	5.6	1.2	1.2	3.4
X	4.3	2.8	3.5	3.8
$\overline{X}$	21.8	13.1	16.2	20.1
SE	4.4	3.0	4.0	4.8

measurement of prednisolone in the dialysate have been taken as the true reference values (Table 2, column "$\overline{Z}_{Ddi}$"). The results calculated from the afferent-efferent concentration differences and blood flow (Table 2, columns $\overline{Z}_{cP}$, $\overline{Z}_{cPK}$ and $\overline{Z}_{cB}$) must be compared with the diffusional loss ($\overline{Z}_{Ddi}$) and not with the total loss ($\overline{Z}_{Dtot}$) of prednisolone, because the equations (Equs. 7, 9 and 11) used for the calculation do not take account of loss by ultrafiltration, since afferent blood flow and efferent blood flow are considered to be identical[1]. Calculation of the mean loss of prednisolone by diffusion on the basis of afferent-efferent plasma concentration differences without considering partitioning of the drug into red blood cells ($\overline{Z}_{cP}$) underestimated the loss by about 40% in all patients (Table 2). If, in addition to the afferent-efferent plasma concentrations, the partitioning is included in the equation ($\overline{Z}_{cPK}$), the mean underestimate of the amount of prednisolone lost was reduced from 40% to 25% ($p < 0.01$; Table 2). Afferent-efferent blood concentration differences ($\overline{Z}_{cB}$) give a better prediction of the amount of prednisolone lost than do plasma levels, with or without corrections for partitioning (Table 2). Calculations based solely on afferent-efferent blood concentration differences underestimate the loss of prednisolone by only 8%.

[1] Equations (7, 9 and 11) may be used to calculate the total loss by adding a term which accounts for ultrafiltration, giving Equ. (4)

Discussion

The most direct technique to assess blood water clearance is to divide the amount of an agent lost each min into the dialysate by the plasma or blood inlet concentration [13]. The clearance values obtained from dialysate flux incorporate both diffusional loss and convective transport by ultrafiltration [13]. The fraction lost by ultrafiltration is very dependent on the pressure gradient across the dialyzer, and changes rapidly within and between patients. The impact of nonlinear plasma protein binding on the removal of prednisolone by diffusion can only be adequately described, therefore, when the fraction lost by ultrafiltration is not included in the clearance calculations. Accordingly, the fraction lost by ultrafiltration was subtracted from the total amount of prednisolone lost into the dialysate by means of Equ. (4).

For most drugs and endogenous compounds the haemodialysis clearance value is a constant, which is independent of the total concentration in plasma. Thus, the concentration-independent and therefore rate-independent clearance value of these substances is useful information with respect to the capacity of a haemodialysis machine to remove them from the circulation [13, 25]. Prednisolone, however, exhibits concentration-dependent clearance, i.e. its clearance is a function of the actual plasma concentration, and so its clearance and plasma concentration must be considered as interdependent variables. The latter clearance values are only useful information when they are given together with the actual plasma concentration of the cleared substance, as shown for the clearance values obtained using total plasma concentrations of prednisolone (Fig. 7).

The metabolic clearance rate of the majority of agents exhibiting concentration-dependent kinetics decreases with increasing plasma concentration, as a consequence of enzyme system saturation [26, 27]. In humans, however, the metabolic clearance rate of prednisolone increases as its total plasma concentration increases [4, 5]. Similarly, the haemodialysis clearance of prednisolone increased with an increasing total plasma concentration of prednisolone (Figs. 6, 7). The underlying mechanism of the concentration-dependent metabolic clearance of prednisolone in humans is not known. One possible explanation of the concentration-dependent metabolic clearance rate of total prednisolone is the non-linear plasma protein binding of prednisolone in humans,

i.e. the free fraction of prednisolone in plasma increases in a non-linear fashion with increasing total plasma concentration of prednisolone. Assuming that the liver or the haemodialysis machine remove only the free fraction of prednisolone, then the total plasma concentration of prednisolone will be a determinant of the total metabolic or haemodialysis clearance rate. The concept that the nonlinear plasma protein binding of prednisolone in humans is responsible for its concentration-dependent metabolic clearance rate is supported by the previous observation that dogs who exhibit virtually no nonlinear plasma protein binding of prednisolone, do not show an increase in prednisolone clearance as total concentration of prednisolone in plasma is increased [15].

The theory that in humans nonlinear plasma protein binding of prednisolone determines the metabolic clearance rate, assumes that the passage time of blood through the liver capillaries is too short for complete dissociation of the drug from transcortin or albumin and for diffusion to the liver cell. If, for the same reason, the haemodialysis clearance were concentration dependent, it would be predicted that 1) the clearance of unbound prednisolone would be a plasma concentration-independent constant, as shown in Fig. 6; and 2) the nonlinear relationship between the total prednisolone concentration in plasma and the amount of prednisolone lost per minute could be adequately described by the equation for nonlinear plasma protein binding (Equ. 14). Our data (Fig. 6) fit that equation; the value of the transcortin capacity and the values of the affinity and capacity of albumin for prednisolone derived for the entire population are within the range observed in stable renal transplant patients, and the value for the affinity of transcortin for prednisolone is slightly too low [5].

There are 3 possibilities to explain why these affinity constants are relatively low: 1) Data from different patients were pooled (Fig. 6). Thus, part of the overemphasized nonlinearity may be the result of interpatient differences in prednisolone binding. 2) The affinity values used above in the comparison were obtained from patients not on haemodialysis and so might be different [5]. 3) From Equ. (14) which relates total concentration to diffusional loss, numerically accurate constants can only be obtained if the entire free moiety from afferent plasma was removed by the passage through the dialyzer. However, as the removal of free prednisolone by simple passage through the machine is incomplete, the values of the affinity constants are expected to differ from those derived from conventional in vitro protein binding studies.

As shown previously [4, 5], and as depicted in Fig. 6, the free fraction of prednisolone increased with increasing plasma concentration of prednisolone. Therefore, as the concentration of prednisolone in plasma rises, a relatively larger fraction of the total concentration will be lost into the dialysate as a consequence of non-linear plasma protein binding (Fig. 6). Interestingly, the fraction of the dose of prednisolone administered which is lost in the dialysate does not increase as the administered dose of prednisolone is increased (Table 1; Fig. 3). This is due to the smaller fraction of the total body prednisolone content entering the dialyzer, as a consequence of the increased volume of distribution with increasing dose of prednisolone (Table 1; Fig. 5). Thus, prednisolone exhibits concentration-dependent haemodialysis clearance, but concentration-independent fractional removal of the dose (Table 1).

Few studies have been reported in which actual measurements have been made in blood and dialysate as well as in plasma to show experimentally the inaccuracy of certain clearance measurements [13, 18]. The present study has demonstrated that dialysis loss, calculated using afferent-efferent differences in plasma concentrations, underestimates the true loss of prednisolone into the dialysate. That was as expected, since for an accurate mass balance the drug in dialysate, in plasma and in red blood cells must all be considered. When concentrations of prednisolone were assessed in whole blood, satisfactory predictions of the loss of prednisolone in the dialysate could be made (Table 2). The predictions were less accurate when based on the partition coefficient, blood flow and afferent-efferent plasma concentrations. The latter method assumes that the partition coefficient measured is representative of the partition coefficient of blood actually equilibrating with dialysate in the haemodialysis machine. This may not be true, as there is an equilibration period of several minutes before the partition coefficient can be assessed, due to the lag-time between blood collection and centrifugation; the equilibration period really available in the dialyser, however, lasts for only a few seconds.

In this kinetic study in 10 patients, 7%–17.5% of a dose of prednisolone was removed during a 5 h dialysis period. We suggest that dosage regimen adjustment may not be necessary for patients undergoing chronic haemodialysis. To predict the amount of a drug with nonlinear plasma protein binding lost in the dialysate, it is mandatory to know afferent-efferent whole blood concentrations and not plasma concentrations, either alone or combined with the partition coefficient. Haemodialysis clearance of such a

drug is concentration dependent, when calculated from total concentrations in plasma, and is concentration-independent when calculated from the of free concentrations.

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Abbreviations Used

C_{Ba} = Afferent blood concentration [ng/ml]
C_{Be} = Efferent blood concentration [ng/ml]
C_D = Dialysate concentration [ng/ml]
C_{Pa} = Afferent plasma concentration [ng/ml]
C_{PaFree} = Free concentration of prednisolone in afferent plasma [ng/ml]
C_{Pe} = Efferent plasma concentration [ng/ml]
CAP_A = Binding capacity of albumin [µg%]
CAP_T = Binding capacity of transcortin [µg%]
Cl_B = Blood clearance [ml/min]
Cl_{Free} = Free plasma clearance [ml/min]
Cl_P = Plasma clearance [ml/min]
H_a = Haematocrit in afferent blood
H_e = Haematocrit in efferent blood
K = Partition coefficient between red blood cells and plasma
KD_A = Dissociation constant of albumin $\left(\frac{M}{L}\right)$
KD_T = Dissociation constant of transcortin $\left(\frac{M}{L}\right)$
Q_B = Blood flow [ml/min]
Q_D = Dialysate flow [ml/min]
Q_F = Flow of ultrafiltrate [ml/min]
t_1 = First sampling time point after haemodialysis started
t_2 = Last sampling time point before haemodialysis was discontinued
Z_{Ddi} = Loss of prednisolone in the dialysate by diffusion [µg/min] ("Z" indicates calculated for each sampling time point)
$\overline{Z}_{Ddi}$ = Mean loss of prednisolone in the dialysate by diffusion [µg/min] ("$\overline{Z}$" indicates calculated for the entire haemodialysis period)
Z_{Dtot} = Total amount of prednisolone recovered in the dialysate [µg/min]
$\overline{Z}_{Dtot}$ = Mean total amount of prednisolone recovered in the dialysate [µg/min]
Z_{Du} = Loss of prednisolone in the dialysate by ultrafiltration [µg/min]
$\overline{Z}_{Du}$ = Mean loss of prednisolone in the dialysate by ultrafiltration [µg/min]
Z_{cB} = Amount of prednisolone lost in the dialysate calculated from afferent-efferent blood concentrations [µg/min]
$\overline{Z}_{cB}$ = Mean amount of prednisolone lost in the dialysate calculated from afferent-efferent blood concentrations [µg/min]
Z_{cP} = Amount of prednisolone lost in the dialysate calculated from afferent-efferent plasma concentrations [µg/min]
$\overline{Z}_{cP}$ = Mean amount of prednisolone lost in the dialysate calculated from afferent-efferent plasma concentrations [µg/min]
Z_{cPK} = Amount of prednisolone lost in the dialysate calculated from afferent-efferent plasma concentrations corrected by the partition coefficient [µg/min]
$\overline{Z}_{cPK}$ = Mean amount of prednisolone lost in the dialysate calculated from afferent-efferent plasma concentrations corrected for the partition coefficient [µg/min]

References

1. Bennett WM, Muther RS, Parker RA, Feig P, Morrison G, Golper TA, Singer I (1980) Drug therapy in renal failure: dosing guidelines for adults. Ann Intern Med 93: 62–89
2. Watanabe AS (1977) Pharmacokinetic aspects of the dialysis of drugs. Drug Intell Clin Pharm 11: 407–416
3. Lewis GP, Jusko WJ, Burke CW, Groves L (1971) The Boston drug surveillance program: Prednisone side-effects and serum protein levels. Lancet 2: 779–781
4. Jusko WJ, Rose JQ (1980) Monitoring prednisone and prednisolone. Ther Drug Mon 2: 169–176
5. Frey FJ, Amend WJC, Lozada F, Frey BM, Holford NHG, Benet LZ (1982) Pharmacokinetics of prednisolone and endogenous hydrocortisone levels in cushingoid and non-cushingoid patients. Eur J Clin Pharmacol 21: 235–242
6. Danon A, Sapira JD (1972) Binding of catecholamines to human serum albumin. J Pharmacol Exp Ther 182: 295–302
7. Westphal U (1966) Steroid-protein interactions. XII Distribution of progesterone and cortico-steroid hormones among serum proteins. Hoppe Seylers Z Physiol Chem 346: 243–256
8. Brodie BB, Lowman EW, Burns JJ, Lee PR, Chenkin T, Goldman A, Weiner M, Steele JM (1954) Observations on the antirheumatic and physiologic effects of phenylbutazone (Butazolidin) and some comparisons with cortisone. Am J Med 16: 181–190
9. Tucker GT, Boyes RN, Bridenbaugh PO, Morre DC (1970) Binding of amidetype local anesthetics in human plasma. I. Relationships between binding, physicochemical properties and anesthetic activity. Anesthesiology 32: 287–303
10. Runkel R, Forchielli E, Sevelius H, Chaplin M, Segre E (1974) Nonlinear plasma levels response to high doses of naproxen. Clin Pharmacol Ther 15: 261–266
11. Meffin PJ, Robert EW, Winkle RA, Harapat S, Peters FA, Harrison DC (1979) Role of concentration-dependent plasma protein binding in disopyramide disposition. J Pharmacokinet Biopharm 7: 29–46
12. Sakiyama R, Pardridge WM (1981) Orosomucoid-bound propranolol is available for transport through the blood brain barrier. Clin Res 29: 278A
13. Gotch FA (1976) Hemodialysis: technical and kinetic considerations. In: Brenner BM, Rector FC (eds) The kidney. WB Saunders, Philadelphia London Toronto, p 1672
14. Frey FJ, Frey BM, Benet LZ (1979) Liquid chromatographic measurement of endogenous and exogenous glucocorticoids in plasma. Clin Chem (NY) 25: 1944–1948
15. Frey FJ, Frey BM, Greither A, Benet LZ (1980) Prednisolone clearance at steady-state in dogs. J Pharmacol Exp Ther 215: 287–291

16. Frey FJ, Lozada F, Guentert T, Frey BM (1981) A single dose of azathioprine does not affect the pharmacokinetics of prednisolone following oral prednisone. Eur J Clin Pharmacol 19: 209–212
17. Gibaldi M, Perrier D (eds) (1975) Pharmacokinetics. Marcel Dekker, New York, p 293
18. Lee CS, Marbury TC, Benet LZ (1980) Clearance calculations in haemodialysis: application to blood, plasma and dialysate measurements for ethambutol. J Pharmacokinet Biopharm 8: 69–81
19. Oppenheimer JH, Schwartz HL, Surks MI (1975) Determination of common parameters of iodothyronine metabolism and distribution in man by non compartmental analysis. J Clin Endocrinol Metab 41: 319–324
20. Benet LZ, Galeazzi RL (1979) Noncompartmental determination of the steady-state volume of distribution. J Pharm Sci 68: 1071–1074
21. Zar JH (ed) (1974) Biostatistical analysis. Prentice Hall Englewood Cliffs, NJ, USA
22. Behm HL, Wagner JG (1979) Errors in interpretation of data from equilibrium dialysis protein binding experiments. Res Commun Chem Pathol Pharmacol 26: 145–160
23. Holford NHG (1979) Multifun. In: Perry HM, Wood JJ (eds) Public procedures notebook. Bolt, Beranek and Newman, Cambridge Mass., USA
24. Putnam FW (1975) The plasma proteins; structure, function and genetic control. Academic Press, New York
25. Gotch FA, Autian J, Colton C, Ginn HE, Lipps BJ, Lowrie E The evaluation of haemodialyzers. US Dept HEW Publication No. N1H-73-103
26. Levy G (1965) Pharmacokinetics of salicylate elimination in man. J Pharm Sci 54: 959–962
27. Gerber N, Wagner JG (1972) Explanation of dose-dependent decline of diphenylhydantoin plasma levels by fitting to the integrated form of Michaelis-Menten equation. Res Commun Chem Pathol Pharmacol 3: 455–460

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