Alterations in prednisolone disposition as a result of time of administration, gender and dose

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- 1 The disposition of total and free prednisolone has been studied in four male and four female volunteers, each of whom received an intravenous dose of 0.075 mg/kg (low) and 1.5 mg/kg (high) of prednisolone at both 06.00 h and 18.00 h.
- 2 For the low dose, free prednisolone clearance was 14% lower ($P = 0.012$) and time-averaged prednisolone free fraction was 22% higher ($P < 0.001$) in the morning, there being no circadian difference in total prednisolone clearance. There was no circadian differences in prednisolone disposition at the high dose. These findings are consistent with a mechanism in which cortisol causes a simultaneous competitive inhibition of prednisolone clearance and plasma protein binding at low, but not at high prednisolone doses.
- 3 Prednisolone clearance was higher in female than in male subjects, the mean increase being 18% ($P = 0.022$) for total prednisolone and 21% ($P = 0.036$) for free prednisolone.
- 4 Mean total prednisolone clearance and steady-state distribution volume were two-fold higher at the high vs the low dose ($P < 0.001$), but free prednisolone clearance showed a dose dependent decrease of 11% ($P = 0.019$). There was no change in free prednisolone steady-state distribution volume.

Keywords prednisolone disposition

Introduction

Circadian variations in the capacity of plasma to bind prednisolone have been reported, in which maximum binding occurred at midnight with a minimum at 08.00 h, and which showed an inverse relationship to plasma cortisol concentrations (Angeli *et al.*, 1978). This report led to the suggestion that circadian variations in cortisol concentrations might produce alterations in prednisolone disposition (Pickup, 1979), but no changes in area under the total prednisolone plasma concentration-time curve were found after oral doses of approximately 0.3 mg/kg given at 08.00 h and 20.00 h (McAllister *et al.*, 1981a). The above authors speculated that circadian alterations in prednisolone disposition might be observed if unbound, rather than total,

prednisolone concentrations were measured. Such an effect could only occur however, due to circadian variations in free prednisolone clearance and would be independent of circadian changes in binding capacity. More recently circadian variation in the area under both total and free prednisolone plasma concentration-time curves have been reported following oral doses of 0.2 mg/kg given at 06.00 h, 12.00 h, 18.00 h and 00.00 h (English *et al.*, 1983). These authors suggested that in addition to circadian variations in prednisolone protein binding, circadian changes in prednisolone clearance occurred similar to those observed with sulphonamides (Dettli & Spring, 1967), with paracetamol and phenacetin (Shively & Vesell, 1975) and with antipyrine (Vesell *et al.*, 1977).

To further examine circadian variations in prednisolone disposition we have carried out a

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study in which a low dose (0.075 mg/kg) and a high dose (1.5 mg/kg) of prednisolone have been administered both at 06.00 h and 18.00 h. In addition, in order to examine gender differences in prednisolone disposition, the study has been carried out in male and female subjects.

Methods

Four male subjects of mean (s.e. mean) age 21.5(1.5) years and weight 71.8(4.4) kg, and four female subjects of mean age 19.8(0.5) years and weight 60.8(3.0) kg, took part in this study. Each subject received a physical examination; a clinical history was taken and a routine biochemical analysis of serum and haematological analysis of blood was carried out prior to the study. Subjects were excluded from the study if they had any of the following: clinical or laboratory evidence of illness, a requirement for regular medication, a history of sensitivity to corticosteroids or of peptic ulcer in the previous 12 months. The nature and purpose of the study was explained to each subject who gave written consent to participation. The study was approved by the institutional committee for human experimentation and complied with the Australian National Health and Medical Research Council's guidelines on human experimentation.

On four separate occasions, 1 week apart, each subject received doses of prednisolone phosphate equivalent to 0.075 (low dose) and 1.5 mg/kg (high dose) of prednisolone between 06.00 h and 06.30 h (morning dose) and between 18.00 h and 18.30 h (evening dose). The doses were administered by constant intravenous infusion into a peripheral arm vein over 5 min. The order of administration of all four doses was randomised. Blood samples (10 ml) were taken from an indwelling cannula before and at 7, 15, 30, 45, 60, 75, 90 and 120 min and then hourly until 9 h after the start of the infusion. The cannula was kept patent by flushing with 1 ml of heparinised saline (5 units/ml) after each sample was taken. Plasma was separated by centrifugation and stored at -20°C until analysed. Subjects were confined to the study room for 9 h after each dose but no attempt was made to influence movement or sleep. A light meal was provided on each occasion 2 h after the dose.

Plasma samples were analysed for prednisolone using a specific high pressure liquid chromatographic (h.p.l.c.) method. Samples were prepared for analysis using the method of Frey *et al.* (1979). Chromatography was carried out using a model M-6000A pump and model 450 u.v. detector (Waters associates) operated at 242 nm. The

column was a 5 μm Lichrosorb Si60 Silica Column, 250 mm long (Merk) and the mobile phase (5% EtOH/95% CH_2Cl_2 - saturated with H_2O) had a flow rate of 2 ml/min (Scott *et al.*, 1980). Under these conditions the retention times for prednisolone, dexamethasone (internal standard), cortisol and prednisolone were respectively 3.37, 4.13, 5.2 and 6.87 min. Calibration curves were constructed by adding known amounts of prednisolone in the range of 20–2000 ng which were analysed concurrently with study samples. The calibration procedure was independent of the volume of plasma in the range 0.1–2.0 ml and the volume of the study samples was varied to stay within the calibration range. The expression: $\log P = 2.603 + 1.016 \log R$ was fitted to data from the 20 calibration curves run during this study ($r^2 = 0.993$, $n = 100$), where P is the amount of prednisolone in the sample and R is the peak height ratio of prednisolone to dexamethasone. The above expression was used to determine the amount of prednisolone in study samples. Calibration procedures similar to those described for prednisolone were established for cortisol.

The free fraction of prednisolone in plasma was measured in all samples following each dose for each subject using equilibrium dialysis. In order to obtain free fractions over the range of total prednisolone concentrations encountered in each study, pre-dose plasma was spiked with sufficient unlabelled prednisolone to ensure equilibrium concentrations equal to or greater than those measured by h.p.l.c. Plasma (1 ml) was dialyzed against an equal volume of isotonic phosphate buffer pH 7.4 at 37°C for 6 h using a spectropor -2 cellulose membrane (Spectrum Medical Industries). [2, 4, 6, 7- ^3H] prednisolone, specific activity 2.22 T Bq/mmol (Amersham) was added in trace amounts (6.4×10^5 d/min, 1.4 ng) in buffer to each half-cell (Dianorm). H.p.l.c. analysis of the [^3H]-prednisolone in which 1 min fractions of effluent were collected for 12 min after injection and counted, showed the presence of only one radioactive peak corresponding to authentic prednisolone. The disintegration rates in aliquots of both sides of the dialysis membrane were determined with an external standard calibration technique and were used to calculate the free fraction (α) of prednisolone at its equilibrium concentration as the quotient of the disintegration rate/ml of buffer and plasma. Equilibrium concentrations (C_e) were determined from the expression (Behm & Wagner, 1981)

$$C_e = \frac{A}{fbV_1 + (1-fb)(V_0 + V_1)}$$

where A is the total amount of drug in the dialysis system, fb is the fraction bound at equilibrium

$(1 - \alpha)$, V_1 is the volume in the plasma half-cell at equilibrium and V_0 is the volume in the buffer half-cell at equilibrium. Mean (s.e. mean) values for V_0 (0.45 (0.02) ml) and V_1 (1.55 (0.03) ml) were determined by weighing the liquid in each half-cell at equilibrium ($n = 20$). Preliminary experiments in which [^3H]-prednisolone was added to either plasma or buffer yielded free fractions which were not significantly different (Mann-Whitney U test, $n_1 = n_2 = 10$, $P > 0.1$), and indicate that equilibrium had been reached. Separate binding curves of free fraction against total prednisolone concentration (C_e) were constructed for each subject and dose. The free fractions corresponding to total concentrations, measured by h.p.l.c., were estimated by interpolation using a cubic spline routine (Knott & Reece, 1971). The free concentrations of prednisolone in study samples were calculated as the product of the total concentration and its corresponding interpolated free fraction. The average plasma prednisolone free fraction over the 9 h sampling period was determined for each subject and dose by constructing a plot of free fraction vs time for each concentration measured by h.p.l.c. The area under this curve was then estimated using the trapezoidal rule and divided by the sampling period to give a time-averaged prednisolone free fraction.

A mono-exponential regression equation was fitted unweighted to the log-linear portion (times > 2 h) of each prednisolone concentration-time profile for both total and free prednisolone concentration in order to estimate the slow rate constant (λ_z). The area under the prednisolone concentration-time curve from zero to infinity (AUC) was calculated using the trapezoidal rule, the area beyond the last concentration measurement being estimated as the quotient of the last concentration and λ_z . Total and free prednisolone clearances were calculated as the dose divided by respective AUC. The total and free distribution

volumes at steady-state were calculated from dose-area relationships using the model independent method of Benet & Galeazzi (1979).

The statistical significance of differences between means for free and total clearance and free and total distribution volume were examined using an analysis of variance with time of prednisolone administration and dose size as repeated measures factors and gender as the non-repeated measures factor (Hull & Nie, 1981). The significance of differences between a single factor, for example, time of administration at the low dose only, was examined using a paired *t*-test.

Results

The age, weight and sex of each subject are listed in Table 1. The derived dispositional parameters for the study are shown for each subject and dose in Figure 1 (total clearance), Figure 2 (free clearance), Figure 3 (total distribution volume) and Figure 4 (free distribution volume).

The results of the analyses of variance are presented in Table 2 as mean values for each dispositional parameter as a factor of time of prednisolone administration, dose size and gender of the subjects. The means in Table 2 have been derived for each factor from all data in these categories. Thus between gender comparisons have been made with data at both doses and both times of administration.

Time of administration

When averaged across both doses and both sexes there were no differences as a result of time of administration for any of the dispositional parameters shown in Table 2. However, if time

Table 1 Subject characteristics

	Male	Weight (kg)	Age (years)
	1	84.6	20
	2	69.7	20
	3	68.6	26
	4	64.2	20
Mean (s.e. mean)		71.8(4.4)	21.5(1.5)
	Female		
	5	56.4	20
	6	61.1	19
	7	69.3	19
	8	56.3	21
Mean (s.e. mean)		60.8(3.1)	19.2(0.5)

Figure 1 Total prednisolone clearance in male (●) and female (▲) subjects for low (0.075 mg/kg) and high (1.5 mg/kg) intravenous doses of prednisolone given at 06.00 h and 18.00 h.

Figure 2 Free prednisolone clearance in male (●) and female (▲) subjects for low (0.075 mg/kg) and high (1.5 mg/kg) intravenous doses of prednisolone given at 06.00 h and 18.00 h.

Figure 3 Total prednisolone steady-state distribution volume in male (●) and female (▲) subjects for low (0.075 mg/kg) and high (1.5 mg/kg) intravenous doses of prednisolone given at 06.00 h and 18.00 h.

Figure 4 Free prednisolone steady-state distribution volume for male (●) and female (▲) subjects given low (0.075 mg/kg) and high (1.5 mg/kg) intravenous doses of prednisolone at 06.00 h and 18.00 h.

dependent changes in prednisolone disposition were due to circadian variation in cortisol plasma concentrations, they might only be observable at the low prednisolone dose where concentrations of cortisol and prednisolone were similar. Mean (s.e. mean) plasma cortisol measurements made just prior to dosing were 132 (14.6) ng/ml, for the

morning dose and 71 (9.7) ng/ml for the afternoon dose ($P < 0.001$). Mean (s.e. mean) plasma prednisolone concentrations 15 min after the low dose were 199 (8.4) ng/ml the corresponding value for the high dose being 2895 (177) ng/ml. For the low doses when plasma cortisol and prednisolone concentrations are similar the prednisolone

Table 2 Effect of gender, dose and time of administration on prednisolone disposition

Factor		Mean value			
Gender (T, D)*	Parameter	Male	Female	P value	
	CL_t ($l\ h^{-1}\ kg^{-1}$)	0.164	0.193	0.022	
	CL_f ($l\ h^{-1}\ kg^{-1}$)	0.620	0.752	0.036	
	Vd_t (l/kg)	0.669	0.676	0.912	
	Vd_f (l/kg)	1.546	1.543	0.988	
Dose (G, T)		Low	High		
	CL_t ($l\ h^{-1}\ kg^{-1}$)	0.116	0.240	0.001	
	CL_f ($l\ h^{-1}\ kg^{-1}$)	0.733	0.655	0.019	
	Vd_t (l/kg)	0.444	0.901	0.001	
	Vd_f (l/kg)	1.457	1.632	0.093	
Time of administration (G, D)		a.m.	p.m.		
	CL_t ($l\ h^{-1}\ kg^{-1}$)	0.178	0.179	0.673	
	CL_f ($l\ h^{-1}\ kg^{-1}$)	0.655	0.718	0.073	
	Vd_t (l/kg)	0.682	0.664	0.667	
	Vd_f (l/kg)	1.537	1.552	0.876	

CL_t = Total clearance

CL_f = Free clearance

Vd_t = Total steady-state distribution volume

Vd_f = Free steady-state distribution volume

T = Time of prednisolone administration (a.m. or p.m.)

D = Prednisolone dose size (high or low)

G = Gender of subjects

* = The factors in parentheses indicate that means have derived from all data in these categories.

Figure 5 Mean prednisolone free fraction-time profiles for all eight subjects after morning (●) and afternoon (▲) intravenous administration of low dose (closed symbols) and high dose (open symbols) prednisolone.

lone free fraction-time profile is higher for the morning than the evening dose (Figure 5). For the high doses which produce plasma prednisolone concentrations approximately 15 times greater than those of cortisol, the prednisolone free fraction profiles are similar at both times (Figure 5). Thus the time-averaged prednisolone free fraction over the 9 h sampling period is elevated by 22% for the morning dose compared to the evening dose for the low dose ($P < 0.001$, Table 2, Figure 6a), but there are no such time dependent differences following the high doses ($P = 0.372$).

If the only effect of the elevated morning plasma cortisol concentrations was to increase the prednisolone free fraction, then an increase in total clearance could be anticipated, similar to that which occurs due to increasing dose (Figure 1). The mean total plasma clearance for the low morning dose of $0.116 \text{ l h}^{-1} \text{ kg}^{-1}$ is not different from that for the evening low dose of $0.117 \text{ l h}^{-1} \text{ kg}^{-1}$ ($P = 0.3$, Table 2), which implies that free prednisolone clearance is reduced. For the low doses, the mean free prednisolone clearance for the morning dose of $0.680 \text{ l h}^{-1} \text{ kg}^{-1}$ is 86% of that for the evening dose (Figure 6b, $P = 0.012$,

Figure 6 (a) Time averaged prednisolone free fraction for morning and evening doses of prednisolone. For the low (0.075 mg/kg) dose the morning free fraction is 22% higher than the afternoon dose ($P < 0.001$), but there is no circadian difference for the high (1.5 mg/kg) dose. (b) Free prednisolone clearance for the low dose is 14% lower in the morning than in the afternoon ($P = 0.012$), there is no circadian difference for the high dose.

Table 2). In contrast there are no time of administration dependent changes in free clearance for the high prednisolone doses (Figure 6b, $P = 0.274$, Table 2).

Dose size

The mean total plasma clearance of prednisolone is approximately two-fold higher for the high dose than the corresponding value for the low dose ($P < 0.001$, Table 2; Figure 1). This increase in total clearance with dose can be ascribed to an increase in free fraction (Figure 5) resulting in the greater availability of unbound prednisolone for clearance. This interpretation is also consistent with the approximately two-fold increase in mean total distribution volume at the high dose compared to the low dose ($P < 0.001$, Table 2; Figure 3). The mean free distribution volume does not differ with dose ($P = 0.093$, Table 2; Figure 4). In contrast with the increase in total clearance with increasing dose, the mean clearance of unbound prednisolone shows a small (11%) but significant decrease with dose size ($P = 0.019$, Table 2, Figure 2).

Gender

The mean total plasma clearance in female subjects is 18% higher than the corresponding mean in male subjects ($P = 0.022$, Table 2; Figure 1). Such an effect could be due to differences either in prednisolone plasma binding or in clearance between the sexes. The mean free prednisolone clearance is 21% higher in female subjects ($P = 0.036$, Table 2; Figure 2) and indicates that the difference between the sexes is due to a difference in clearance rather than in plasma protein binding. This interpretation is consistent with the lack of change in total distribution volume ($P = 0.912$, Table 2; Figure 3) which would be sensitive to changes in plasma protein binding. The free distribution volume is also similar in both sexes ($P = 0.988$, Table 2; Figure 4).

Discussion

In vitro studies of prednisolone-cortisol binding interactions have demonstrated that these two compounds can mutually displace each other from transcortin but prednisolone has a 2.5-fold higher affinity than cortisol for this protein (Rocci *et al.*, 1982). The same authors also showed that cortisol is unable to alter the binding of prednisolone to albumin as the binding affinity for prednisolone to albumin is 300 times that of cortisol. At plasma prednisolone concentrations of approximately 200 ng/ml or less, where bind-

ing to transcortin predominantly determines the prednisolone free fraction, circadian variations of plasma cortisol concentration could give rise to alterations in prednisolone free fraction although these effects should be modest given that peak morning plasma cortisol concentrations are approximately 150–200 ng/ml and afternoon nadir values are 30–50% of this peak (Angeli *et al.*, 1878, Umeda *et al.*, 1981). At plasma prednisolone concentrations greater than 200 ng/ml, where albumin binding predominantly determines the free fraction, circadian variations in plasma cortisol concentrations could be expected to produce minimal alterations in prednisolone free fraction.

The above reports and that showing a circadian variation in free prednisolone clearance (English *et al.*, 1983) led us to formulate the following hypotheses regarding circadian variations in prednisolone disposition.

- (1) If circadian variations in plasma cortisol gave rise to alterations only in prednisolone plasma protein binding then:
 - (a) circadian changes in total prednisolone clearance and distribution volume should be observable at low but not at high prednisolone doses;
 - (b) such circadian alterations in total clearance and distribution volume should not occur for free clearance and distribution volume irrespective of dose.
- (2) If in addition to changes in prednisolone plasma protein binding, there were similar changes in prednisolone elimination (free clearance) then:
 - (a) circadian variations in prednisolone total clearance may not be observable due to the opposing effects of increased prednisolone free fraction and the decreased free clearance;
 - (b) If a decrease in prednisolone free clearance occurred as a result of a general circadian alteration in drug metabolising capacity, it should be observable for both high and low prednisolone doses;
 - (c) If such a decrease in free clearance were the result of competitive inhibition of prednisolone and cortisol clearance then it may only be observable at low prednisolone doses when plasma concentrations of cortisol and prednisolone were similar.

Time of administration

The approximately two-fold circadian variation in plasma cortisol concentrations produces effects

on prednisolone plasma binding which are consistent with those reported by others (Rocci *et al.*, 1982). Thus for the low dose, cortisol is able to displace prednisolone from binding sites but this effect is a weak one, however at the high dose cortisol does not displace prednisolone from plasma binding sites (Figures 5 and 6a).

Cortisol and prednisolone differ in structure only by the presence of a double bond in ring A and are thought to undergo a number of common metabolic transformations (Fotherby & James, 1972). Competitive inhibition of one or more of these metabolic transformations may be the mechanism of the circadian reduction in the clearance of unbound prednisolone at low doses. Such a mechanism would not operate to the same extent at high prednisolone doses due to the relative concentrations of cortisol and prednisolone. The circadian variation in free prednisolone clearance for the low but not for the high dose (Figure 6b) supports a mechanism involving competitive inhibition of cortisol and prednisolone metabolism rather than a more general effect. This mechanism is also consistent with a greater reduction of prednisolone free clearance by oral contraceptives at low prednisolone doses than at high prednisolone doses, given that cortisol concentrations are elevated approximately two-fold in subjects taking oral contraceptives (Meffin *et al.*, 1984).

At low prednisolone doses the overall effect of circadian variation in plasma cortisol concentrations on prednisolone disposition is the result of two separate phenomena; displacement of prednisolone from plasma binding sites with a resulting increase in free fraction, and a simultaneous inhibition of free prednisolone clearance of similar degree. The net effect of these two processes is an unchanged total prednisolone clearance.

English *et al.* (1983) recently reported the mean area under the total plasma prednisolone concentration-time curve following oral prednisolone administration at 18.00 h was 73% of that at 12.00 h, although there were no differences in this parameter for 06.00 h or 00.00 h administration compared with 12.00 h. Such a result could occur if the inhibitory effect of cortisol on prednisolone free clearance was greater than that on prednisolone free fraction; but this possibility cannot be assessed further as plasma cortisol concentrations were not reported. In the above study the morning area under the free prednisolone concentration-time curve was 157% of the corresponding evening measurement. The magnitude of this effect is approximately 2.5-fold that found in the present study although plasma prednisolone concentrations are similar in the two studies. The difference in

magnitude of circadian alteration of unbound prednisolone clearance between the two studies may indicate that there is circadian variation in prednisolone bioavailability.

The reported circadian variation in prednisolone serum binding (Angeli *et al.*, 1978) has led to speculation regarding circadian variation in prednisolone disposition (Pickup, 1979; McAllister *et al.*, 1981a). Because during continuous therapy, the free concentration of a drug in plasma is determined only by dose rate and free clearance, changes in plasma protein binding will not alter the concentration of physiologically active unbound prednisolone. The magnitude of the morning decrease in free clearance reported in the present study of 14% is modest but the more substantially decreased free prednisolone clearance of 36% reported after oral doses (English *et al.*, 1983) may be of greater consequence. It should be stressed that this effect is largest for small doses that produce plasma prednisolone concentrations comparable to those of peak morning cortisol concentrations, and is not discernible at higher prednisolone doses (Figure 6b). A decrease in morning prednisolone free clearance may produce consistently elevated free prednisolone concentrations in patients on low maintenance doses who receive morning rather than evening doses and who have intact circadian cortisol production. In spite of this it has been shown that less suppression of hypothalamic-pituitary-adrenal function occurs with morning rather than evening doses (Nichols *et al.*, 1965; Seigre & Klaiber, 1966). If it is presumed that there is no morning loss of sensitivity in anti-inflammatory or other desired clinical response, then these findings add an additional factor in support of the common clinical practice of morning rather than evening doses as such a regimen will not only produce less adrenal suppression but also result in more anti-inflammatory response than the same dose given in the evening.

Dose

The dose dependent changes in total clearance (Figure 1) and total distribution volume (Figure 2) are similar to those reported by others and have been attributed to non-linear binding of prednisolone to plasma proteins (Rose *et al.*, 1980; McAllister *et al.*, 1981b; Legler *et al.*, 1982). In contrast other studies have failed to demonstrate dose dependent increases in total clearance and distribution volume (Al-Habet & Rogers, 1980) or have attributed the non-linear disposition of prednisolone to reasons other than saturable plasma protein binding (Tanner *et al.*, 1979).

It has recently been reported that the reversible conversion of prednisolone to prednisone appears to follow Michaelis-Menten kinetics, in which prednisone concentrations reach a maximum of approximately 60 ng/ml, the K_m for this process being approximately 260 ng/ml of prednisolone (Legler *et al.*, 1982). In spite of this finding the above authors reported a dose dependent increase in prednisolone free clearance, but commented that this increase in apparent prednisolone free clearance could not be unambiguously attributed to a change in clearance processes until unbound prednisone and prednisolone concentration measurements were available. In addition, Wagner *et al.* (1981) have pointed out that because of the interconversion of prednisone and prednisolone, clearance calculations based on dose/area relationships can be interpreted differently depending on which particular metabolic model of prednisolone biotransformation is correct. As the exact relationship between prednisone and prednisolone interconversion and the subsequent metabolism of these compounds has not been established, the significance of these dose dependent changes in apparent free prednisolone clearance cannot be determined. Over the 20-fold dose range of this study there is a small (11%) but significant ($P = 0.019$, Table 2) decrease in free clearance. Such a dose dependent decrease in free clearance has not previously been reported but has also been observed by us in a similar study (Meffin *et al.*, 1984). Such an effect could be an artifact resulting from inappropriately measured prednisolone free fractions, but the lack of a dose dependent change in free distribution volume makes this less likely. The difference in dose dependent clearance reported in the present study and by Legler *et al.* (1982) may be due to the differences in dose as the plasma prednisolone concentrations produced by the high dose in the present study were more than 10 times those produced by the high dose in the study of Legler *et al.* (1982).

Gender

The clearance of total and free prednisolone is approximately 20% higher in females than in males but there is no gender dependent difference in total or free distribution volume (Table 2). Boekenoogen *et al.* (1983) recently reported no significant gender dependent differences in any of the parameters of prednisolone disposition for five female and eight male control subjects. None of the female control subjects in either study were taking oral contraceptives which have been shown to decrease prednisolone free clearance (Boekenoogen *et al.*, 1983; Meffin *et al.*, 1984). The reason for this difference is unclear but similar higher clearances have been demonstrated for female subjects with desmethyldiazepam and diazepam and the metabolic clearance of antipyrine to norantipyrine, 4-hydroxyantipyrine and 3-hydroxymethylantipyrine (Allen *et al.*, 1980; Greenblatt *et al.*, 1980; Teunissen *et al.*, 1982), but the demethylation of chlordiazepoxide is higher in males (Roberts *et al.*, 1979).

Conclusion

We have demonstrated that in addition to the concentration dependent changes in prednisolone disposition due to non-linear protein binding there are alterations in prednisolone disposition as a result of dose, time of administration and gender which interact in a complex manner. The data from this study indicate that on average a female subject receiving a small dose of prednisolone in the evening would have a free clearance approximately 30% higher than a male subject receiving a dose of the same size in the morning. These differences are small but may be important for optimizing the doses of patients maintained on low doses of prednisolone.

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