Pharmacokinetics of prednisone and its metabolite prednisolone in children with nephrotic syndrome during the active phase and in remission

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- 1 The kinetics at steady state of prednisone and its metabolite prednisolone were determined in nine nephrotic children during the active phase of the disease and in remission.
- 2 There were no differences in serum prednisone levels between the two occasions. Prednisone levels were lower than prednisolone levels.
- 3 Total serum prednisolone levels were significantly lower during the active phase than in remission (AUC: 2452 ± 207 vs 3392 ± 293 ng ml⁻¹ h respectively). Half-life values were similar on both occasions.
- 4 The binding of prednisolone to serum proteins was markedly impaired during the active phase as compared to the remission. Free fraction values correlated positively with total drug concentration. A negative correlation between free fraction and serum albumin level was found during the active phase.
- 5 Free prednisolone levels during the active phase did not differ significantly from those observed during remission (AUC: 937 ± 128 vs 847 ± 81 ng ml⁻¹ h respectively).
- 6 These data indicate that pharmacokinetic changes are unlikely to be responsible for alterations in steroid responsiveness in nephrotic patients with hypoalbuminaemia.

Keywords prednisone prednisolone pharmacokinetics nephrotic syndrome

Introduction

Prednisone and prednisolone have established themselves as first-line drugs for the treatment of idiopathic nephrotic syndrome of childhood. It is a common clinical observation, however, that the degree of therapeutic response and the incidence of side effects may show considerable interindividual variation among patients receiving 'standard' daily doses (Jusko & Rose, 1980). Although this variability can be explained to some extent by differences in severity of the disease, pharmacokinetic factors might also be important. If this is the case, their characterization would provide a more rational approach to the individualization of drug regimens.

While there is some evidence that the disposition of prednisone and prednisolone may be altered in nephrotic patients as compared to normal volunteers (Frey & Frey, 1982a), the possibility of pharmacokinetic changes in relation to the regression of the disease during prolonged administration has been little investigated. In a recent study, Rocci *et al.* (1982) were able to demonstrate that the volume of distribution and the plasma clearance of prednisolone are abnormally high during the active phase of the nephrotic syndrome but tend to decrease as the disease improves. Regrettably, the clinical relevance of these findings is difficult to assess

because measurements of the unbound (pharmacologically active) plasma concentration of the drug were not performed in these patients. This information is particularly critical in this kind of investigation, because the protein binding of prednisolone is highly dependent on plasma protein levels which, in turn, are known to increase markedly within a few weeks of therapy in responsive patients.

The present study was designed to evaluate prospectively the pharmacokinetics of prednisone and prednisolone (including the plasma protein binding of prednisolone) in children with steroid-sensitive nephrotic syndrome (SSNS). To this purpose, each child was studied on two occasions: (1) during the active phase of the disease and (2) during remission.

Methods

Patients

Nine children (see Table 1 for details) were included in the study after obtaining informed consent from the parents and, whenever applicable, from the patients themselves. Each child had been admitted to hospital within a few days of relapse of SSNS. Diagnosis was based on clinical and laboratory findings and on the history of steroid responsiveness. Six children were not receiving any drug at the time of admission; three children (cases 1, 4 and 7) were on maintenance therapy with a low to moderate dose of prednisone (30 mg on alternate days). Patients who had taken other drugs during the previous 2 months were excluded.

Protocol

All patients were started on therapy with high-dose prednisone (Deltacortene, Schering), approximately 2 mg/kg given as a single daily dose at 08.00 h orally for 4 weeks. Four patients were also given frusemide during the first 3–5 days (Table 1). After 4 weeks the dosage of prednisone was gradually tapered down according to clinical response. No drugs other than frusemide and prednisone were given on a regular basis during the first 4 weeks of therapy. Each child was studied twice, 3–5 days after starting high-dose prednisone (during the active phase) and 20–25 days later (during remission). Due to the short elimination half-life of prednisone and its metabolite prednisolone both occasions reflect steady-state conditions, thus making comparison of data easier. Blood

samples (5 ml) were obtained on each occasion just before the dose (time 0) and again 2.5, 5, 7.5, 10 and 24 h later. The serum was kept frozen at -20°C and analyzed within 4 months.

Analytical methods

The concentration of prednisone and prednisolone in serum and serum dialysates was determined on a Perkin-Elmer Series 3B high-performance liquid chromatograph according to the method described by Rose & Jusko (1979). Serum albumin concentrations were measured by radial immunodiffusion. The binding of prednisolone to serum proteins was determined by equilibrium dialysis on a Dianorm apparatus (Innovativ Medizin Esslingen, Zürich) using 1 ml-compartment Teflon cells and open Visking tubing (thickness 0.025 mm) as semipermeable membrane. Since the volume of blood that could be obtained from each child was limited, experiments were performed with 0.5 ml serum samples which were brought to 1.0 ml by diluting with 0.13 M phosphate buffer pH 7.4. One ml diluted serum containing a trace amount of $[6,7\text{-}^3\text{H}]\text{-prednisolone}$ (20000 d/min, specific activity 45 Ci/mmol, source: Radiochemical Centre, Amersham) was dialyzed for 6 h against 0.02 M PIPES Na buffer, pH 7.4. During dialysis the cells were placed in a water bath at 37°C and rotated at 30 rev/min. After the attainment of equilibrium conditions, the radioactivity in the serum (d/min_s) and in the buffer (d/min_b) compartments of the dialysis apparatus was determined on a Packard Tri-Carb Model 3330 liquid scintillation spectrometer. The free fraction of prednisolone (α) was determined according to the formula $\alpha = (d/\text{min}_b)/(d/\text{min}_s)$. The free concentration was calculated by multiplying the total concentration value by α . The values of free prednisolone fraction (α) calculated by the above method refer to the ratio between the drug concentrations in the two compartments of the dialysis apparatus. This does not correspond precisely to the free fraction in the original sample because of the initial dilution and because of the transfer of drug and water across the semipermeable membrane during dialysis. These processes may influence the free fraction in a complex fashion (Riva *et al.*, 1982). Since the plasma protein binding of prednisolone is saturable (concentration dependent), the decrease in prednisolone concentration in the serum compartment may result in underestimation of the original free fraction. On the other hand, the reduction in protein concentration caused by the initial dilution and by the osmotic drive of water to the serum compartment during dialysis may lead to a somewhat decreased binding capacity.

Table 1 Details of the patients included in the study

Patient	Sex	Age (years)	Disease state	Weight (kg)	Serum albumin (g l ⁻¹)	Associated therapy	Prednisone dosage (mg day ⁻¹)
1	M	10	AP R	38.8 37.2	12 31	Frusemide 25 mg three times daily	60 60
2	M	6	AP R	20.0 21.4	17 23	—	40 40
3	M	11	AP R	33.9 35.0	11 31	—	70 70
4	F	6	AP R	28.2 30.0	12 39	—	55 55
5	M	11	AP R	40.0 40.5	28 40	—	70 70
6	M	4	AP R	20.3 20.0	19 29	—	40 40
7	M	7	AP R	22.4 23.0	10 22	Frusemide 25 mg twice daily	40 40
8	M	10	AP R	35.2 33.1	13 19	Frusemide 25 mg twice daily	60 60
9	M	15	AP R	52.3 48.8	16 27	Frusemide 30 mg twice daily	60 60
Mean ± s.e. mean		8.9 1.1	AP R	32.3 ± 3.6 32.1 ± 3.2	15 ± 2 29 ± 2	—	55 ± 4 55 ± 4

AP = active phase. R = remission.

Whilst these opposing factors may not cancel out each other, the lack of a precise correspondence between values of free fraction *in vivo* and *in vitro* should not invalidate the comparability of the results in the present study conditions.

Pharmacokinetic parameters

The following parameters were taken into consideration: times of peak ($t_{\max}$), peak concentration values ($C_{\max}$), first-order rate constants of the terminal (elimination) phase (β), elimination half-lives ($t_{1/2}$) and areas under the concentration curves (AUC). β was calculated by linear regression from log-concentration values over the terminal monoexponential phase of the curve. $t_{1/2}$ was calculated as $0.693/\beta$. AUCs were calculated by the trapezoidal rule.

Statistical analysis

Comparisons between parameters determined in the two study occasions were made by using the Student's *t*-test for paired data. Correlations were assessed by means of linear regression analysis.

Results

Clinical details

During the active phase of the disease all children showed extensive proteinuria, hypoalbuminaemia and oedemas of variable degree. Four weeks later all patients were in remission as indicated by the disappearance of the oedemas and of the proteinuria and a rise in serum albumin (Table 1). Changes in body weight did not show any consistent trend between the two study occasions. Serum creatinine, BUN, glucose, GOT, GPT, alkaline phosphatase and haematology tests were within normal limits in all subjects.

Pharmacokinetics of prednisone

Pharmacokinetic parameters derived from serum prednisone values are given in Table 2. Peak prednisone levels were obtained in most cases at 2.5 h after dosing and were usually below 100 ng/ml; at 24 h serum levels had usually declined below 10 ng/ml. In most patients half-life values could not be reliably determined due to irregularities in the concentration profile. At all sampling times there were no significant differ-

Table 2 Pharmacokinetic parameters of prednisone during the active phase (AP) and in remission (R). For abbreviations see **Methods**

Subject	Disease state	$t_{\max}$ (h)	$C_{\max}$ (ng ml ⁻¹)	AUC (ng ml ⁻¹ h)
1	AP	2.5	61	839
	R	2.5	60	786
2	AP	5	57	738
	R	7.5	46	647
3	AP	5	116	899
	R	5	55	662
4	AP	2.5	42	567
	R	2.5	48	761
5	AP	5	42	619
	R	5	35	483
6	AP	2.5	39	336
	R	2.5	57	466
7	AP	5	45	512
	R	2.5	51	472
8	AP	2.5	57	410
	R	2.5	65	721
9	AP	2.5	30	281
	R	2.5	47	458
Mean $\pm$ s.e. mean	AP	3.6 ± 0.4	55 ± 8	578 ± 72
	R	3.6 ± 0.6	51 ± 3	606 ± 45
<i>P</i>		NS	NS	NS

Table 3 Pharmacokinetic parameters of prednisolone during the active phase (AP) and in remission (R). Unless indicated otherwise, values are derived from total plasma concentration data. For abbreviations see **Methods**

Subject	Disease state	$t_{\max}$ (h)	$C_{\max}$ (ng ml ⁻¹)	$C_{\max}^{\text{free}}$ (ng ml ⁻¹)	k_e (h ⁻¹)	$t_{1/2}$ (h)	AUC (ng ml ⁻¹ h)	AUC_{free} (ng ml ⁻¹ h)
1	AP	2.5	345	218	0.20	3.2	2154	964
	R	2.5	343	104	—	—	3400	769
2	AP	5	311	106	0.27	2.6	2537	648
	R	5	417	104	0.17	3.9	4149	792
3	AP	2.5	452	293	0.28	2.4	3063	1642
	R	2.5	415	192	0.30	2.3	2358	794
4	AP	2.5	463	169	0.18	3.8	3164	1038
	R	2.5	532	187	0.25	2.8	3670	1018
5	AP	5	364	125	0.20	3.3	3186	724
	R	5	384	117	0.17	4.0	4179	907
6	AP	2.5	409	162	0.36	1.9	2077	599
	R	2.5	380	196	0.38	1.8	2136	390
7	AP	2.5	354	223	0.27	2.5	2608	1443
	R	2.5	527	358	0.24	2.9	2945	1283
8	AP	2.5	334	204	0.27	2.6	1857	836
	R	2.5	627	175	0.23	3.0	4754	947
9	AP	2.5	225	97	0.24	2.8	1424	538
	R	2.5	445	140	0.27	2.6	2936	720
Mean ± s.e. mean	AP	3 ± 1	362 ± 24	177 ± 21	0.25 ± 0.02	2.8 ± 0.2	2452 ± 207	937 ± 128
	R	3 ± 1	452 ± 31	175 ± 26	0.25 ± 0.02	2.9 ± 0.2	3392 ± 293	847 ± 81
P		NS	< 0.05	NS	NS	NS	< 0.05	NS

ences in serum levels between the active phase and the remission state. AUCs values were almost identical on the two occasions and were consistently much lower than those calculated for the metabolite prednisolone (Tables 2 and 3).

Pharmacokinetics of prednisolone

The serum level profile of total and free prednisolone is illustrated in Figure 1. Peak levels of total prednisolone were usually achieved at 2.5 h and were invariably much greater than the levels of the parent drug. At all sampling times (except for 0 and 24 h, when the concentration of the metabolite was undetectable in most cases) total prednisolone levels were significantly ($P < 0.05$) lower during the active phase than in remission. AUC values of total drug were increased by about 50% during remission, while no difference was seen in half-life values between the two occasions (Table 3).

When free rather than total prednisolone was considered, a completely different pattern emerged. Free prednisolone levels in fact did not

Figure 1 Levels of free (Δ , $\blacktriangle$) and total ($\circ$, $\bullet$) prednisolone levels during the active phase of the nephrotic syndrome (open symbols) and in remission (closed symbols). Values represent the means $\pm$ s.e. mean.

show any consistent change between the two study occasions. There was a tendency for the levels of free drug to be increased during the active phase of the disease (with a more than 50% increment in AUC values in two subjects), but the difference failed to reach statistical significance. In all patients, the time course of the free levels mirrored the time course of the total levels, with a peak at 2.5 h and a rapid decline thereafter.

The observation that during the active phase of the disease total prednisolone levels were decreased while free levels did not change significantly implies that the protein binding of the metabolite was reduced in this condition. Since prednisolone binding varies depending on drug concentration, plots of free fraction vs total concentration were compared (Figure 2). In all subjects, highly significant positive correlations were found (r values ranging from 0.84 to 0.99). As illustrated in Figure 2, however, the regression lines for the data sets in the active phase of the disease were consistently shifted to the left. This indicated that, at any given prednisolone concentration the unbound fraction was greater during active disease than in remission. The free prednisolone fraction at a concentration of 250 ng/ml (calculated from the equations of the regression lines) is reported in Table 4; it can be seen that during the active phase of the disease the values are twice as high as those observed during remission. There was a significant inverse relationship between the percent changes in free prednisolone fraction (data from Table 4) and the percent changes in total prednisolone AUC between the two study occasions ($r = -0.81$, $P < 0.01$). As illustrated in Figure 3, there was also an inverse correlation between the free fraction of prednisolone and the concentration of albumin in the patients' serum during the active phase of the disease ($r = -0.71$, $P < 0.05$). This correlation, however, was no longer apparent during remission ($r = 0.09$, NS).

Discussion

Although prednisone is widely used in therapeutic practice, the drug itself is said to be devoid of glucocorticoid effect (Sarett *et al.*, 1963; Editorial, 1979) and to exert its action after conversion to its hydroxylated active metabolite prednisolone, whose concentration in plasma exceeds that of the parent drug. The findings obtained in the present study indicate that there are important changes in the kinetics of metabolically derived prednisolone between the acute phase of the nephrotic syndrome and its remission. The decrease in total prednisolone levels observed

Figure 2 Relationship between free prednisolone fraction and total prednisolone concentration during the active phase (open symbols) and in remission (closed symbols) in six representative cases. Prednisolone values on the abscissa refer to the concentration of the drug in the undiluted sample before dialysis (the numbers in the left corners represent the code identification number of each patient as given in Table 1).

Table 4 Free fraction of prednisolone during the active phase (AP) and in remission (R). Free fractions were calculated from the regression lines shown in Figure 3 and refer to the prednisolone concentration of 250 ng/ml in the undiluted sample prior to dialysis

Subject	Free prednisolone fraction (%)	
	AP	R
1	51	23
2	29	16
3	47	31
4	31	23
5	27	20
6	27	18
7	57	32
8	50	16
9	45	23
Mean $\pm$ s.e. mean	40 $\pm$ 4	22 $\pm$ 1
	$P < 0.001$	

during active disease could be due to (a) impaired gastrointestinal absorption of prednisone, (b) impaired conversion of prednisone to prednisolone, (c) enhanced activity of the enzymes responsible for prednisolone catabolism, (d) decreased protein binding with consequent larger volume of distribution and enhanced total plasma clearance or (e) a combination of the above factors. Reduced absorption or lesser conversion are most unlikely because both the total serum concentration of the parent drug and the free concentration of the metabolite remained relatively similar on both study occasions. Moreover, previous studies have demonstrated that neither of these processes is impaired in patients with acute nephrotic syndrome (Rocci *et al.*, 1982). Enhanced enzyme activity leading to increased prednisolone elimination is also unlikely because such event would be expected to reduce the area under the free concentration curve of the metabolite, which was clearly not the case. The

Figure 3 Relationship between free prednisolone fraction at 250 ng/ml (data from Table 4) and serum albumin concentration during the active phase (—, open symbols) and in remission (---, closed symbols). Values on the abscissa refer to the serum albumin concentration in the undiluted sample before dialysis.

observed kinetic changes therefore can be ascribed to the marked impairment in serum protein binding during the acute phase of the disease. This was indirectly confirmed by the significant correlation between decrease in free prednisolone fraction and change in total prednisolone AUC between the two study occasions. This interpretation is also consistent with the previous finding of an increased volume of distribution of prednisolone in nephrotic children in active disease as compared to the remission state (Rocci *et al.*, 1982), even though in that study no significant differences in AUC were seen between the two occasions. In those children plasma protein binding measurements were not performed, thus making interpretation of data largely speculative.

The marked concentration-dependency of prednisolone binding to serum proteins (Jusko & Rose, 1980) was clearly confirmed in all of our patients. This phenomenon is related to saturation of the high affinity-low capacity binding to transcortin, while the binding to albumin shows low affinity and high capacity and appears to be largely concentration independent (Lewis *et al.*, 1971; Wagner *et al.*, 1981; Steele *et al.*, 1982). The mechanism responsible for the marked impairment of prednisolone binding capacity in the acute phase of the nephrotic syndrome remains to be established. Our finding of a significant negative correlation between albumin concentration and free prednisolone fraction during acute disease does not prove a cause-effect relationship. Transcortin, for example, has been considered in some studies as a more important

determinant of changes in prednisolone binding (Frey & Frey, 1982b; Steele *et al.*, 1982) and is known to decrease in nephrotic patients in a parallel fashion with the fall in plasma albumin (Frey & Frey, 1982a). The possible role of endogenous binding inhibitors should also be considered.

From the clinical point of view, the most important finding of the present study is the demonstration that the changes in total prednisolone concentrations were not accompanied by any consistent alteration in the levels of free drug. This observation highlights the limitations of using total drug levels as a measure of pharmacological effect when changes in plasma protein binding are involved (Perucca & Richens, 1980). Since only the free concentration is considered to be in equilibrium with the drug molecules at the receptor sites, our data can be taken as evidence that, despite the increase in total prednisolone concentration, the degree of pharmacological response should not be enhanced as treatment is continued.

In previous studies, reduced albumin levels have been correlated with increased incidence of prednisolone adverse effects (Lewis *et al.*, 1977; Uribe *et al.*, 1977) and a reduction in prednisolone dosage has been recommended based on serum albumin concentration (Centeno *et al.*, 1978). This increased toxicity in hypoalbuminaemia has been considered to result from an elevated free fraction of the steroid in plasma but, at least for patients with normal liver function, this hypothesis appears to be an incorrect extrapolation of *in vitro* data to the *in vivo* situa-

tion. We could confirm that hypoalbuminaemia is associated with a marked rise in free prednisolone fraction. However, it is the free *concentration* and not the free *fraction* that determines the pharmacological effect. Since in patients with normal hepatic function the clearance of prednisolone is directly related to the free fraction in plasma (Pickup, 1979), any impairment in binding would be expected to result in increased metabolic elimination and reduced total drug concentration without appreciable changes in the concentration of free drug. This was indeed the case in most of our patients, in whom the free concentration during the active phase of the disease did not differ from that observed during remission, when the hypoalbuminaemia had largely resolved. Only two patients, for un-

known reasons, behaved differently and exhibited a net (> 25%) elevation of the free concentration during the active phase.

Taken altogether, our data are in accordance with those of a recent study in which hypoalbuminaemic nephrotic patients were found to have lower total prednisolone levels but similar free drug levels as compared to normal volunteers (Frey & Frey, 1982a). In agreement with Frey & Frey (1982a), we can conclude that there is no pharmacokinetic basis for anticipating a consistent alteration of response in nephrotic patients with hypoalbuminaemia. If a greater susceptibility of glucocorticoid-related side effects exists in these patients, this could involve an increased sensitivity of the target tissues to steroid stimulation.

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