Prednisolone protein binding in renal transplant patients

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Prednisolone pharmacokinetics and protein binding characteristics were studied in 10 renal transplant patients with various degrees of renal function (serum creatinine: 80–380 $\mu\text{mol/l}$) who received their usual oral maintenance dose of prednisolone ($0.18 \pm 0.04 \text{ mg/kg}$). Plasma was assayed for prednisolone and hydrocortisone by h.p.l.c. and free prednisolone concentrations were determined in each sample by a rapid ultrafiltration technique. Free prednisolone area under curve (AUC_u) ranged from 101 to 436 $\text{ng ml}^{-1} \text{ h}$ and was 6.3 to 15.0% of total prednisolone AUC. The fraction AUC_u/AUC was closely related to serum albumin and creatinine concentrations determined at the time of study (multilinear regression correlation coefficient $r^2 = 0.830$, $P < 0.0001$); elevated serum creatinine and low albumin concentrations were associated with a higher % free. These results suggest that much of the variability in prednisolone protein binding could be attributed to inter-patient variability in serum albumin and creatinine concentrations. Total prednisolone concentrations would be potentially misleading in any comparisons made between patient groups with different renal function.

Keywords prednisolone protein binding renal transplantation

Introduction

Prednisolone is a synthetic glucocorticoid which binds to both albumin and transcortin in plasma (Rocci *et al.*, 1982). Albumin has a low affinity but high capacity for binding, and transcortin a high affinity but low capacity. Saturation of transcortin binding capacity, which occurs at usual therapeutic doses and results in concentration-dependent binding of the drug (Sandberg *et al.*, 1957; Frey *et al.*, 1982), is reflected in an increase in prednisolone free fraction with increasing total prednisolone concentration. At low prednisolone concentrations, hydrocortisone also plays a role in prednisolone binding by competing for transcortin binding sites (Rocci *et al.*, 1982). The net result is that prednisolone is more than 90% bound at low concentrations and approximately 60% bound at high concentrations.

The relationship between prednisolone protein binding and kidney function in renal transplant patients has not been investigated. Renal impairment is often associated with alterations in the protein binding of drugs (Gibson, 1981) though decreased protein levels and changes in binding affinity. In the present study, a specific h.p.l.c. assay and a rapid ultrafiltration technique were employed to investigate prednisolone protein binding in renal transplant patients with various degrees of renal function.

Methods

Patients

Ten renal transplant patients (eight males, two females) of mean age ($\pm$ s.d.) of 39 ± 13 years

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and weight 73 ± 15 kg were studied. All had normal liver function and none was receiving contraceptive medication. Serum creatinine levels in these patients ranged from 80–380 $\mu\text{mol/l}$ (mean $\pm$ s.d., 212 ± 95) on the day of the pharmacokinetic study. Creatinine clearance, calculated according to Lott & Hayton (1978), was 55 ± 28 ml/min. Renal function was stable in all but patients 3, 4 and 7, and patient 5 had slight proteinuria. Each patient was studied after their usual morning oral dose of prednisolone (10–20 mg, 0.18 ± 0.04 mg/kg). Other medication remained unchanged at the time of the study and consisted mainly of azathioprine, atenolol, prazosin, dipyrimadole and cimetidine or ranitidine. Patients fasted from midnight prior to the study and did not receive any methyl-prednisolone therapy for 2 days prior to the study. Blood samples were taken from an indwelling catheter, 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h post-dose. The plasma was separated and stored at -20°C to await analysis.

Prednisolone assay and protein binding

Total prednisolone and hydrocortisone concentrations were determined by high performance liquid chromatography using an improved sample extraction procedure (Stewart *et al.*, 1982) and chromatographic conditions described by Frey *et al.* (1979).

Free prednisolone concentrations were determined by ultrafiltration using disposable MPS-1 devices designed by Amicon (Danvers, MA, USA). The devices have been applied to the measurement of free hydrocortisone levels (MacMahon *et al.*, 1983) and a similar procedure was adopted for prednisolone. Five microlitres of labelled tracer {2,4,6,7- ^3H } prednisolone (50–75 Ci/mmol) in toluene/ethanol (9:1) (Amersham Int. Inc., Amersham, Buckinghamshire, England) was dried in a disposable glass tube under nitrogen. Plasma (1.2 ml) was added to the residue and the tube vortexed. The mixture was equilibrated at 21°C for 30 min. One millilitre of this sample was then pipetted into the filtration device which was centrifuged at 1000 g for 30 min in a 34° fixed-angle rotor maintained at $21 \pm 2^\circ\text{C}$. Duplicate 50 μl samples were then counted. Reproducibility of the technique was checked at total plasma concentrations of 50 and 500 ng/ml ($n = 5$).

Data analysis

Model independent methods were used to determine drug half-life and area under curve (AUC) (Gibaldi & Perrier, 1982). Since prednisolone

free fraction in plasma was dependent on drug concentration and therefore varied over the post-dose period, the expression $\text{AUC}_f/\text{AUC} \times 100$ (% free) was used to describe the time averaged free fraction within a patient. Prednisolone binding parameters were determined using methods described by Frey & Frey (1984) and a nonlinear regression analysis program (BMDPAR, 1979). To assess the relationship between various clinical and prednisolone binding variables and prednisolone % free, multilinear regression analysis was carried out using a Hewlett-Packard HP-85B desk-top computer. The significance of the contribution of each variable to the relationship was tested using the generated F value.

Results

Ultrafiltration of plasma samples containing prednisolone at concentrations of 50 and 500 ng/ml gave 9.2 ± 0.2 and $27.8 \pm 0.3\%$ unbound, respectively. These values were similar to those obtained by Bergrem (1983) using equilibrium dialysis at 37°C and concentrations of 50 ($12.3 \pm 0.4\%$) and 500 ng/ml ($29.6 \pm 0.5\%$). The difference at the lower prednisolone concentration may reflect the temperature dependency of transcortin binding. Plasma protein concentrations may have also been different in these studies. Correlation with equilibrium dialysis was acceptable in patient samples ($r = 0.93$, $n = 23$, $P < 0.01$). Recovery measurements showed that prednisolone binding to the filter membranes was negligible.

Prednisolone plasma concentrations peaked 1.5 ± 1.1 h post-dose (range 0.5–4.0 h) with total concentrations of 353 ± 144 ng/ml (184–585) and free concentrations of 64.2 ± 27.1 (40.4–106). AUC was 2310 ± 820 ng ml $^{-1}$ h (1350–3800) and AUC_f 218 ± 96 ng ml $^{-1}$ h (101–436). Hydrocortisone levels ranged from 0 to 436 ng/ml (mean 94.3 ± 125) immediately prior to drug administration ($t = 0$).

Albumin binding capacity ranged from 32 to 46 g/l (mean $\pm$ s.d., 40 ± 4.1) and was not related to serum creatinine ($r^2 = 0.017$, $P > 0.1$) or creatinine clearance ($r^2 = 0.280$, $P > 0.1$). Transcortin binding capacity was more variable (range 17 to 48 mg/l; mean $\pm$ s.d., 33 ± 13) as were the association constants for albumin (range $1.0\text{--}6.1 \text{ M}^{-1} \times 10^{-3}$; mean $\pm$ s.d., $2.6 \pm 1.8 \times 10^{-3}$) and transcortin (range $2.2\text{--}20.8 \text{ M}^{-1} \times 10^{-7}$; mean $\pm$ s.d., $8.0 \pm 5.3 \times 10^{-7}$). Again there were no significant relationships between these parameters and serum creatinine or creatinine clearance. Prednisolone % free

ranged from 6.3–15.1 (mean $\pm$ s.d., 9.6 ± 3.1) and was related to albumin concentration alone ($r^2 = 0.578$, $P < 0.05$) but not to serum creatinine ($r^2 = 0.356$, $P > 0.05$), or creatinine clearance ($r^2 = 0.368$, $P > 0.05$) alone. There was no relationship between zero-time hydrocortisone plasma levels and % free. Multilinear regression analysis using the independent variables serum creatinine, creatinine clearance, albumin and transcortin binding capacities (concentrations) and albumin and transcortin affinity constants with the dependent variable, prednisolone % free gave $r^2 = 0.960$ ($P < 0.0001$) although the only significant contributors to this relationship were serum creatinine and albumin concentrations. Serum creatinine and albumin concentration together accounted for 83.0% of the variability in prednisolone % free ($P < 0.0001$).

Discussion

Pharmacokinetic parameters obtained for both free and total prednisolone in this study were comparable to those observed by others (Bergrem, 1983; English *et al.*, 1983; Frey *et al.*, 1981, 1982; Frey & Frey, 1984; Gambertoglio *et al.*, 1982; Nelson *et al.*, 1982; Ost *et al.*, 1984). Binding parameters were also similar to those obtained in other renal transplant patients with

the exception of mean albumin binding capacity which was about twice that observed by Frey *et al.* (1981). The value obtained in the present study was closer to that reported for the control group in their study. Transcortin affinity constants also tended to be higher in the present study although there were larger inter-patient differences in these values. Albumin affinity constants were, however, comparable in both studies and not significantly different from control values.

This study has shown that much of the variability in prednisolone protein binding observed in renal transplant patients could be attributed to inter-patient variability in serum albumin and creatinine concentrations. Albumin concentrations alone made the most substantial contribution reflecting the fact that albumin binding capacity was an important determinant of prednisolone free fraction. Serum creatinine, but not creatinine clearance, was also a significant contributor and may reflect elevated concentrations of one or more other solutes which could inhibit prednisolone binding in renal impairment.

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