Prednisolone Replacement Therapy Mimics the Circadian Rhythm More Closely Than Other Glucocorticoids

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Background: This study examined the pharmacokinetic profile of prednisolone.
Methods: Using a newly developed ultra-performance liquid chromatography MS/MS method, prednisolone profiles in healthy volunteers and patients with adrenal insufficiency already treated with prednisolone were prospectively analyzed in a tertiary center.
Results: Twelve prednisolone day curves were analyzed. Six patients with secondary adrenal insufficiency provided 7 curves and 3 healthy volunteers provided 5 curves, 1 of which was with the prednisolone administered in divided doses. The mean prednisolone dose required for adequate replacement in hypoadrenal patients was 3.86 mg. The overall mean maximal serum concentration ($C_{\text{max}}$) was 114.0 μg/L and was achieved at an average time to maximal concentration ($T_{\text{max}}$) of 1.43 h. Total glucocorticoid exposure was represented by the mean area under the curve to 24 h (AUC$_{0-24h}$), which was 518.2 μg·h/L. Splitting the dose substantially increased the total glucocorticoid exposure.
Conclusions: The pharmacokinetic profile of prednisolone is similar to the published profile of dual-release hydrocortisone. Once-daily prednisolone can thus be used as a replacement for hydrocortisone. Further studies need to be carried out to accurately calculate an equivalent replacement dose. Prednisolone levels are a useful adjunct to dose adjustment when low doses are being used for replacement.

IMPACT STATEMENT

All patients who require steroid therapy are potential beneficiaries. Those with adrenal or pituitary failure are most likely to benefit because their current steroid replacement is suboptimal. Levels of hydrocortisone have been used by many clinics to optimize dosage, but dosing thrice daily makes patients’ adherence to the planned levels difficult. Prednisolone levels decline more slowly than hydrocortisone levels, and prednisolone replacement can also be optimized in a similar fashion. An important advancement is the determination of the equivalence of hydrocortisone with prednisolone. Prednisolone has a larger effect than previously recognized.

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Nonstandard abbreviations: AUC, area under the curve; UPLC, ultra-performance liquid chromatography; LOQ, limit of quantification; AUC$_{0-24h}$, area under the curve to 24 h; $C_{\text{max}}$, maximal serum concentration.
Adrenal insufficiency is treated with glucocorticoid replacement and has been proven to reduce the high mortality of this condition (1). Nevertheless, even with appropriate therapy, patients have a shorter life expectancy by 12 years. This mortality gap may in part be due to unrecognized or inappropriate replacement, exposing patients to the long-term risks of osteoporosis, diabetes, cancer, and cardiovascular disease (2, 3). Up to 15% of deaths in this group are due to Addisonian crises (4).

The accepted principle of glucocorticoid replacement is to attempt to mimic the normal cortisol circadian rhythm while exposing the patient to the minimum possible levels of exogenous glucocorticoid with an overnight nadir. The objective is to minimize the short-term symptoms of insufficiency and prevent the onset of a crisis while avoiding the long-term adverse effects of excessive replacement with steroids. Early observations suggested that following the physiological cortisol profile using 3 daily doses of hydrocortisone promotes better symptom control without increasing cumulative daily steroid exposure (5). More recently, tighter control of serum cortisol concentrations has been achieved using subcutaneous hydrocortisone infusions (6). This scenario has allowed for even closer adherence to the physiological profile and has proven that it is possible to adequately control symptoms with lower systemic steroid exposure than previously achievable.

Achieving optimal replacement with oral therapy has proven to be a substantial challenge. Hydrocortisone remains the most frequently prescribed glucocorticoid for this purpose, but owing to its relatively short half-life of 113 min, multiple daily doses are required and patients often experience trough steroid levels during the day that are below the levels required for replacement (7). To overcome this, a dual-release hydrocortisone has been designed to emulate a physiological profile with a single dose (8). However, the suggestion that this improves glycemic and hemodynamic markers may have been due to the reduced steroid exposure experienced by the dual-release hydrocortisone group, as demonstrated by the lower area under the curve (AUC)4 (9).

Prednisolone has a longer half-life than hydrocortisone, permitting a once-daily administration to give adequate glucocorticoid replacement while mimicking the circadian cortisol rhythm (10). With a structure similar to cortisol, the C1-C2 double bond confers an extended half-life and increased potency. Prednisolone is traditionally understood to be 4 times more potent than hydrocortisone, suggesting that a dose of 5 mg daily would be equivalent to 20 mg daily of hydrocortisone (11). However, concerns about the metabolic side effects of using doses of prednisolone of 5 mg daily have limited its use as a replacement therapy to the minority of patients who do not tolerate multiple-dose hydrocortisone replacement. Using patients with congenital adrenal hyperplasia, prednisolone has been shown to have a potency 6–8 times greater than hydrocortisone (12), which suggests that too large a dose of prednisolone has been used in the past. Esteban et al. (13) suggested that the cortisol production rate in healthy volunteers is 9.9 mg ± 2.7 mg/day when produced by endogenous adrenal glands. The correct replacement dose when given as divided doses is likely to be higher than this, because of the first-pass metabolism effects on oral hydrocortisone. In addition, splitting the dose increases the overall exposure compared to a single dose (11), so that the pulsatile secretion from the endogenous adrenal may be more effective than single oral doses. Patients are often given replacement doses of 15–30 mg daily, and if 20 mg hydrocortisone is the correct replacement dose, prednisolone doses of 2.5–3.3 mg daily may be more appropriate.

The ability to measure cortisol and undertake cortisol day curves has helped in prescribing the correct replacement dose of hydrocortisone (2). The lack of such assays for prednisolone may have been a barrier to prescribing replacement doses.
of prednisolone, which has traditionally been used in much higher doses for immunosuppression and for its antiinflammatory action, rather than as a replacement therapy.

Using ultra-performance liquid chromatography (UPLC) MS/MS, we performed day curves on patients and healthy volunteers to assess the feasibility of using prednisolone as a glucocorticoid replacement.

**METHOD**

Healthy volunteers were recruited to take between 3 and 4 mg prednisolone and gave informed consent. One volunteer had 3 curves examining the absorption of prednisolone in the various circumstances. One curve was done completely fasted, and a second was carried out after a normal breakfast 2 h before prednisolone administration. The third curve was completed taking prednisolone in divided doses: 2 mg at 8 AM followed by a further 1 mg at 12 noon. Routinely performed day curves for patients who were already stable on prednisolone as replacement for secondary adrenal insufficiency were also reviewed. None were on any mineralocorticoid replacement.

Blood samples were taken via an indwelling intravenous catheter, inserted before administration of prednisolone, and samples were taken at fixed time points. Adequacy of prednisolone replacement at our institution is judged by a measured 8-h serum prednisolone level of between 10 and 20 μg/L.

Blood samples were collected into SST BD Vacutainer tubes, containing serum clot activator and a serum separating gel. All specimens were spun and separated within 4 h, and serum was stored at −20 °C before analysis. All samples from an individual prednisolone day curve were analyzed within the same analytical run.

**Analytical methods**

Prednisolone concentrations were determined by UPLC-MS/MS (Waters Quattro Premier Mass Spectrometer). Deuterated prednisolone (D6) was used as internal standard (CDN isotopes). A total of 60 μL of a 120 μg/L stock internal standard solution was added to 20 μL patient sample or standard and vortex-mixed for 20 s. Proteins were precipitated with 20 μL 0.5 mol/L zinc sulfate and 300 μL 50% methanol, and the supernatant was injected into a Waters HSS T3 column (1.8 μm, 2.1 × 50 mm). Chromatography was performed using mobile phases A and B, consisting of 2 mmol/L ammonium acetate and 0.1% formic acid in water and 2 mmol/L ammonium acetate and 0.1% formic acid in methanol, respectively. At a flow rate of 0.5 mL/min, the solvent program used was 55% mobile phase B for 1.5 min, followed by 95% phase B for 1 min and 55% phase B for 1 min. The total run time was 3.5 min per sample.

Analysis on the mass spectrometer was performed in positive ion mode using electrospray ionization. Ion transitions of 361→147 (quantifier) and 361→343 (qualifier) were used to detect prednisolone, and 363→150 was used for D6-prednisolone.

A least squares regression calibration curve was drawn at the beginning of each run using 6 standard calibrants of known concentration. These were prepared in PBS with 0.1% BSA. The concentrations were 0, 40, 125, 250, 500, and 1000 μg/L. QC was performed at 3 levels using patient serum pools at 3 mean concentrations: 25.5, 79.1, and 123.5 μg/L. QC samples were analyzed at the beginning and end of each batch of patient samples. Levey Jennings charts were prepared based on the mean and SDs derived from at least 30 QC values obtained from separate analytical batches. QC performance was monitored using the Unity software program (Bio-Rad), and patient results were deemed acceptable if all QC values were within the limits.
of ± 2 SD. Specimen calibration curves and chromatograms are shown in Fig. 1.

Full method validation was conducted according to the guidelines of Honour (14), including precision, linearity, interference testing, and determination of limit of quantification (LOQ).

In the absence of a gold standard reference measurement procedure, accuracy was assessed by determining the mean of spike and recovery experiments. Prednisolone at 3 different concentrations was spiked into 5 different patient samples. Pre- and postextraction spikes were compared to assess the recovery of prednisolone during the extraction procedure.

Quantitative assessment of matrix effects was conducted according to the method of Matuszewski et al. (15). This approach compares both the analyte and internal standard signals obtained in the presence and absence of matrix. Prednisolone was spiked at 3 different concentrations into solvent and into 3 separate postextraction samples. This process was repeated for the D6-prednisolone internal standard. This quantitative approach to the assessment of matrix effects is

**Fig. 1.** (A), Typical chromatogram of a patient sample showing the multiple reaction monitoring (MRM) transitions for the quantifier (top pane) and qualifier (middle pane) ions for prednisolone and for the internal standard (lower pane). (B), Typical calibration curve of standards prepared in PBS with 0.1% BSA. In both A and B, the response is the peak area ratio of prednisolone/D6-prednisolone.
superior to the qualitative approach, whereby blank samples are injected into the mass spectrometer during continuous infusion of either analyte or internal standard.

Analytical carryover was assessed using samples with high and low concentrations of prednisolone. Five replicates of the low sample were run consecutively, followed by five replicates of alternating high (H) and low (L) samples (i.e., LLLLLHLHLHL HLHL). The initial low sample replicates were then compared to the low samples that followed high samples.

**Statistical analysis**

Measured prednisolone concentrations were plotted against time to produce prednisolone day curves. Data were collated and apparent terminal elimination half-lives were calculated using Microsoft Excel 2010. All graphs, areas under the curve to 24 h (AUC_{0–24h}), and pharmacokinetic data were created using GraphPad Prism 6 (GraphPad Software).

To permit analysis of the patient group data, it was assumed that prednisolone was completely eliminated by 14 h after the dose was administered. This result was concordant with the expected prednisolone concentration at 14 h, calculated by extrapolating the terminal half-life determined from previous data points and preliminary day curves in several individuals, which demonstrated full clearance by 14 h.

**RESULTS**

**UPLC-MS/MS method**

Intraassay and interassay CVs were 2.7% and 4.1%, respectively, at a prednisolone concentration of 50 μg/L. Assessment of precision at 400 and 750 μg/L yielded both intra- and interassay CVs <6.5%. The method was linear up to 5000 μg/L, with a lower LOQ of 10 μg/L (CV 20% and measured value within ±10% of the expected value). Signal-to-noise ratio at the lower LOQ was 26. Quantitative assessment of recovery and matrix effects both yielded results within the 100 ± 20% limits of acceptability. For the spike and recovery experiments, the mean recovery observed was 92.6% (range 83.1%–104%) and, for the quantitative assessment of matrix effects, the mean response in the presence of matrix as a percentage of the signal obtained in absence of matrix was 100% (range 96%–105%).

Interference testing with supraphysiological quantities of fludrocortisone (1000 μg/L), dexamethasone (1000 μg/L), corticosterone (1154 nmol/L), 6α methyl-prednisolone (396 μg/L), cortisol (1109 nmol/L), 21-deoxycortisol (1154 nmol/L), and prednisone (333 μg/L) showed no interference.

No significant carryover was detected between injected samples containing high concentrations of prednisolone and injected samples containing low concentrations of prednisolone.

**Baseline characteristics**

Demographic data can be found in Table 1. Eleven day curves were performed on single daily prednisolone dosing and were available for pharmacokinetic analysis. One curve was completed with split dosing of prednisolone.

**Pharmacokinetic data**

The individual plasma concentration time profiles are charted in Fig. 2, and the mean values are found in Table 2. The average replacement dose of prednisolone used in patients in this study was 3.86 mg (range 3–5 mg), corresponding to a maximal serum concentration (C_{max}) of 109.9 μg/L, which was achieved at 1.56 h in the patient group. In the healthy volunteer group, a mean of 3.75 mg prednisolone was used, corresponding to a C_{max} of 121.4 μg/L at an average time of 1.21 h. The mean terminal half-life of prednisolone was calculated to be 2.54 h (range 1.75–3.75 h). The mean AUC_{0–24h} was 518.2 μg·h/L.
When the prednisolone dose was divided, the AUC_{0–24h} increased substantially (Fig. 2B). A single dose of prednisolone of 4 mg corresponded to an AUC_{0–24h} of 426.2 μg |·| h/L. When 3 mg was administered as a split dose in the same participant (2 mg at 8 AM and 1 mg at 12 noon), this corresponded to an AUC_{0–24h} of 473.8 μg|·|h/L. The terminal half-life of prednisolone was conserved in individuals who had more than 1 day profile, despite the differences in the administered dose. The half-life of prednisolone was 2.22 h after 4 mg (fasted) and 2.29 h after 4 mg was administered 2 h after breakfast, and 2.23 h on the 2 mg + 1 mg split regimen in the same volunteer.

### Adverse effects

No adverse effects were reported during this study.

### DISCUSSION

We have determined plasma prednisolone concentrations in both healthy volunteers and a cohort of patients using UPLC-MS/MS. This technique was used to characterize prednisolone day curves and better study its pharmacokinetic profile.

An in vitro study by Lan et al. (16) demonstrated the dissociation constant (K_d) of cortisol and prednisolone to be 61 and 27 nmol/L, respectively, corresponding to a 2.26-fold difference in receptor binding affinity. The combination of increased receptor binding affinity and the discrete pharmacokinetic profile of prednisolone with the longer half-life than hydrocortisone would both contribute to an increased dose ratio when compared to hydrocortisone.

The traditional view is that the prednisolone to hydrocortisone bioequivalence ratio is 1:4 (11). This view is based on the antiinflammatory action of steroids seen at higher doses (12) and may be influenced by exogenous corticosteroids binding serum proteins in a nonlinear fashion (17). With increasing serum concentrations of prednisolone, there is a larger fraction of free prednisolone, promoting greater body clearance (18).

Caldato et al. (12) conducted a study on 2 cohorts of patients with congenital adrenal hyperplasia. The group discovered that prednisolone dosed on average at 2.6 mg/m^2 sufficiently suppressed serum androgen precursors and was equivalent to thrice-daily hydrocortisone dosed at 14.9 mg/m^2. This result suggests that the conversion ratio between prednisolone and hydrocortisone is somewhere between 6:1 and 8:1.

<table>
<thead>
<tr>
<th>Table 1. Demographics and baseline characteristics.</th>
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<tbody>
<tr>
<td><strong>Healthy volunteers (n = 3)</strong></td>
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<td>------------------------------------------</td>
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<tr>
<td><strong>Average age, years (range)</strong></td>
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<tr>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
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<tr>
<td><strong>Number of day curves</strong></td>
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<tr>
<td><strong>Replacement dose</strong></td>
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<tr>
<td>2 mg and 1 mg</td>
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<tr>
<td><strong>Divided doses</strong></td>
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<td>3 mg</td>
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<tr>
<td>4 mg</td>
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<td>5 mg</td>
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^a Results are n (%) for categorical data, unless otherwise stated.
Fig. 2. (A), Amalgamation of prednisolone profiles from healthy volunteers. (B), Amalgamation of prednisolone profiles from the patient group. (C), Single prednisolone profile of 1 healthy volunteer taking 4 mg in a single daily dose and 3 mg in split doses (2 mg at 8 AM and 1 mg at 12 noon).
Prednisolone is an attractive alternative for glucocorticoid replacement therapy. Once-daily administration may promote better compliance in light of many patients omitting the second and third doses of thrice-daily regimens (8), and Johannsson et al. (9) also found a decided patient preference for once-daily regimens vs thrice-daily regimens. It is, however, the perception that prednisolone confers a higher risk of adverse metabolic effects that has proven the biggest obstacle to its widespread use. In particular, there is concern about the high incidence of osteoporosis in prednisolone-treated patient groups. These observations have been made from studies comparing 7.5 mg prednisolone to 30 mg hydrocortisone, working on the premise that there is 4:1 bioequivalence (19, 20). In the light of our findings, it is likely that the prednisolone doses used in these studies were excessive. In our clinical experience, it is seldom that a prednisolone dose as high as 5 mg is required to achieve adequate glucocorticoid replacement, with the majority of patients only requiring 3 or 4 mg. Higher doses of hydrocortisone have long been associated with suppressed bone formation (21), and by extension, it is likely that over-replacement with prednisolone will have the same effect.

Data from patients who have undergone multiple profiles on different prednisolone doses confirm that the half-life ($T_{1/2}$) is conserved, although interindividual variation ranged from 1.75 to 3.75 h. In our clinical experience, measuring 8-h serum prednisolone concentrations for a given dose and aiming for an 8-h level of 10–20 μg/L currently offers a surrogate indicator of adequate glucocorticoid replacement, and our current data suggest that this correlates to an acceptable $AUC_{0–24h}$. The majority of patients are likely to only require a single 8-h postdose level to assess the suitability of a given prednisolone dose. If there is any difficulty in interpreting the results, there remains the recourse of performing a full prednisolone day profile.

Splitting the prednisolone dose may substantially affect the total steroid exposure by increasing the $AUC$. Johannsson et al. (9) found a similar large increase in the $AUC$ when hydrocortisone was administered thrice daily. It is important that patients realize the impact of changing the timing of their doses. Many patients who forget a dose of a drug simply take two doses together; this will result in a less bioavailable steroid than if the tablets are taken on time.

### Table 2. Pharmacokinetics and dose results.

<table>
<thead>
<tr>
<th></th>
<th>Healthy volunteers (mean)</th>
<th>Adrenal insufficiency (mean)</th>
<th>Total (mean)$^a$</th>
<th>Healthy volunteer, divided doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$, μg/L</td>
<td>121.4 (15.7)$^b$</td>
<td>109.9 (48.0)</td>
<td>114.0 (38.6)</td>
<td>94.1</td>
</tr>
<tr>
<td>$C_{8h}$, μg/L</td>
<td>15.3 (4.4)</td>
<td>18.9 (3.1)</td>
<td>17.6 (3.8)</td>
<td>26.3</td>
</tr>
<tr>
<td>$AUC$, μg · h/L</td>
<td>488.0 (107.8)</td>
<td>535.5 (197.7)</td>
<td>518.2 (165.9)</td>
<td>473.8</td>
</tr>
<tr>
<td>$T_{\text{max}}$, h</td>
<td>1.21 (0.25)</td>
<td>1.56 (0.56)</td>
<td>1.43 (0.47)</td>
<td>1.0</td>
</tr>
<tr>
<td>Average dose, mg</td>
<td>3.75 (0.5)</td>
<td>3.86 (0.52)</td>
<td>3.82 (0.48)</td>
<td>3 (2 mg and 1 mg, split)</td>
</tr>
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</table>

$^a$ Total mean calculations exclude “Healthy volunteer, divided doses” values.

$^b$ Results are represented as mean (SD).

$^c$ $C_{8h}$, concentration at 8 h; $T_{\text{max}}$, time to maximal concentration.

**CONCLUSION**

A simple and robust UPLC-MS/MS method for the measurement of serum prednisolone has been developed and validated. This method enables accurate day curve monitoring and dose titration in patients with adrenal insufficiency on prednisolone. Prednisolone is superior to the
Prednisolone as Glucocorticoid Replacement

The current standard of thrice-daily hydrocortisone at mimicking the physiological cortisol profile and, with a once-daily administration, may promote better compliance. Traditionally, its bioequivalence to hydrocortisone has been quoted at 1:4, although there is some evidence that the true figure is closer to somewhere between 1:6 and 1:8 at physiological doses (12). Together with our data, this suggests that a dose of between 3 and 5 mg prednisolone once daily is likely to be adequate glucocorticoid replacement for most patients.

Further prospective randomized and blinded studies are required to determine which regimen achieves optimum well-being with minimal long-term effects on bone and glycemc control.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

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