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Modified-release hydrocortisone to provide circadian cortisol profiles

Short title: Circadian hydrocortisone therapy

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Disclosure statement

MD, CG, MC, JN-P, KD, WA have nothing to declare. DPM received research funds from Phoqus Plc. RR & AR have equity interests in (Diurnal Ltd). HH is employed as a consultant for Diurnal Ltd.

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Precis: A modified release formulation of hydrocortisone that allows for delayed and sustained release of hydrocortisone can potentially replicate normal un-stressed physiological cortisol levels.

Abstract

Context: Cortisol has a distinct circadian rhythm regulated by the brain's central pacemaker. Loss of this rhythm is associated with metabolic abnormalities, fatigue and poor quality of life. Conventional glucocorticoid replacement cannot replicate this rhythm.

Objectives: To define key variables of physiological cortisol rhythm and by pharmacokinetic modelling test whether modified-release hydrocortisone (MR-HC) can provide circadian cortisol profiles.

Setting: Clinical Research Facility.

Design and Methods: Using data from a cross-sectional study in healthy reference subjects (n=33) we defined parameters for the cortisol rhythm. We then tested MR-HC against immediate-release (IR-HC) in healthy volunteers (n=28) in an open-label, randomised, single-dose, crossover study. We compared profiles to physiological cortisol levels, and modelled an optimal treatment regimen.

Results: The key variables in the physiological cortisol profile included: peak 15.5mcg/dL (95% reference range 11.7–20.6), acrophase 0832h (95%CI 0759h–0905h), nadir<2mcg/dL (95% reference range 1.5-2.5), time of nadir 0018h (95%CI 2339h–0058h), and quiescent phase (below the mesor) 1943h to 0531h. MR-HC 15mg demonstrated delayed and sustained release with a mean (SEM) C_{max} of 16.6 (1.4) mcg/dL at 7·41 (0·57) hrs after drug. Bioavailability of MR-HC 5, 10 & 15mg was 100, 79, & 86% that of IR-HC. Modelling suggested that MR-HC 15 to 20mg at 2300h and 10mg at 0700h could reproduce physiological cortisol levels.

Conclusion: By defining circadian rhythms and using modern formulation technology it is possible to allow a more physiological circadian replacement of cortisol.

Introduction

Cortisol secretion follows a distinct circadian rhythm, with circulating levels low at sleep onset, beginning to rise between 0200h and 0400h, peaking within an hour of waking and then declining through the day (1). This circadian rhythm is determined by the central endogenous clock (pacemaker) of the hypothalamic-pituitary-adrenal (HPA) axis, located in the hypothalamic supra-chiasmatic nucleus (SCN), which drives release of corticotropin releasing hormone (CRH), in turn leading to secretion of adrenocorticotropin (ACTH) from the pituitary and thus cortisol from the adrenal.

The central SCN clock has an approximate period length of 24·2 hours, and requires daily adjustment by photoperiod to synchronize to the 24 hour day/night cycle. In addition to the central clock, there are molecular oscillators (peripheral clocks) in most mammalian cells (2). The phase of these peripheral clocks is reset by signals from the central pacemaker. The specific signals from the central to peripheral clocks have not been fully established. Circadian gene expression can be induced by serum shock (3), and glucocorticoids are able to phase delay or advance peripheral oscillators (4). Thus cortisol appears to act as one secondary messenger from central to peripheral clocks.

The HPA axis plays an important role in maintaining alertness and modulating sleep (5). Conditions associated with insomnia including depression, sleep apnoea, and chronic fatigue, disrupt the circadian rhythm of cortisol leading to metabolic abnormalities and increased cardiovascular risk (6, 7). Patients with adrenal insufficiency have loss of the normal circadian rhythm of cortisol and excess mortality mainly from cardiovascular events and infections (8-10). This may be explained in part by the fact that current replacement regimens cannot replace the normal physiological rhythm of cortisol (11). Moreover, with current replacement regimes the

majority of patients with adrenal insufficiency report impaired health-related quality of life (HR-QOL) (12, 13), and early morning fatigue (14, 15) with subsequent socioeconomic health problems. Hence, physiological cortisol replacement in patients with adrenal insufficiency may be advantageous when compared to conventional treatments.

Infusions of hydrocortisone (cortisol) can mimic the normal circadian rhythm of cortisol and improve biochemical control and quality of life in patients with adrenal insufficiency (16, 17). Since infusions are not a practical solution, we designed a modified release hydrocortisone (MR-HC). A small proof of concept study confirmed the potential utility of the formulation to reproduce the normal overnight rise in cortisol levels (18).

We have now analyzed circadian cortisol levels from a group of healthy subjects to define the parameters of physiological cortisol secretion. These can then be used to judge any new therapy that aims for physiological circadian replacement of cortisol. We have then performed a detailed phase 1 study on new modified-release formulations of hydrocortisone (MR-HC) and compared them to the kinetics of 10mg immediate-release hydrocortisone (IR-HC), the conventional hydrocortisone preparation currently used in routine clinical care for adrenal insufficiency and congenital adrenal hyperplasia. These data were then used to define optimal treatment regimens with MR-HC for mimicking physiological cortisol levels, a novel concept in the delivery of glucocorticoid replacement.

Subjects and Methods

Healthy reference group

33 normal individuals who had undergone detailed, 24-hour, 20-minute, cortisol profiling provided data for definition of the physiological cortisol circadian rhythm (Table 1) (19). This

data set was used as all individual measurements were available to us and the data was generated with the same modern cortisol assay. To further validate that this data set is representative of the general population all mean (±95% confidence intervals (CI)) cortisol concentrations, $AUC_{(0-24)}$ and time variables obtained from the healthy reference group were compared to similar variables derived from the previously published literature (20-29).

Defining physiological ranges

Cortisol levels generally show a skewed distribution. Therefore, in defining physiological ranges cortisol levels were transformed to the natural logarithm enabling the cortisol geometric mean to be calculated at each time point. 95% CI were calculated for: the $AUC_{(0-24)}$ (area under the curve from time 0 to 24 hours), peak cortisol, trough cortisol and 24-hour mean cortisol. In addition we report 95% reference ranges (±2SD) for the peak and trough cortisol.

Cosinor analysis

For each individual cortisol profile, a cosinor model with a second harmonic was fitted to the data (30). A group cosinor model was computed by averaging the coefficients from the individual fits. Circadian timing estimates were obtained for each individual cortisol profile. This allowed us to calculate the mesor (rhythm adjusted mean), the acrophase (time of peak in rhythm), the nadir (lowest point of the rhythm), and the quiescent phase (start, taken as the time when the cortisol level was equal or less to the mesor for more than an hour, and end, when the cortisol level was equal or more to the mesor for more than an hour). In a previous study reporting the quiescent phase, the cutoff for the start and end was taken as 5 mcg/dL (138nmol/l) (21), a very similar value to the mesor 5.2 mcg/dL (143·6nmol/l) that we obtained for use in this study.

Pharmacokinetic Analysis for modified-release hydrocortisone

32 healthy male subjects were recruited. Entry criteria: 18 to 50 years; no illness, operation or steroid use in the previous three months and no regular medication. The study was approved by

the Plymouth Independent Ethics Committee, UK and all subjects gave informed written consent. The sampling was performed in the Chiltern Clinical Research Unit, UK.

Dose-response study

20 subjects were randomized to receive three of the following four single-dose regimes: 5mg MR-HC (1x5mg), 15mg MR-HC (1x15mg), 30mg MR-HC (2x15mg), 10mg immediate release hydrocortisone (IR-HC) with a one week washout between treatments. 12 other subjects were randomized to receive either 10mg (2x5mg) of MR-HC or 10mg IR-HC with a one-week washout period.

The MR-HC or IR-HC dose was taken at 2200h. Participants had their HPA axis suppressed with 1mg oral dexamethasone at 1800h and 2200h on Day 1 and then at 0600h, 1200h and 1800h on Day 2 during each treatment period. Plasma ACTH levels were taken at 2155h on Day 1 and on 0600h on Day 2 whilst serum cortisol levels were taken prior to ingestion of the drug and were repeated every 30 minutes for the first four hours, then at 0200h, 0300h, 0400h, 0500h, 0600h, 0800h, 1000h, 1300h, 1600h, 2200h (Table 2).

MR-HC

The tablet has an insoluble barrier coat protecting all but the upper face of the tablet. The unprotected face exposes a delaying layer that slowly erodes in the small intestine to present the sustained release drug-containing layer. Two different dose units of MR-HC were available, 5mg and 15mg. MR-HC was supplied by Phoqus PLC (Kent, UK).

IR-HC

10mg tablets were from MSD (Hertfordshire, UK). A 10mg dose was used to avoid un-physiological peak values that exceed the binding capacity of cortisol binding globulin which in

our view would complicate the analysis when comparing PK values to a modified release formulation of hydrocortisone (31).

Assays

Serum cortisol was measured using the Bayer Advia Centaur Automated Immunoassay System. The inter-assay coefficient variation was 7% at 7·2μg/dl (200nmol/l), and 8% at 38μg/dl (1,050nmol/l). Plasma ACTH was measured using the DPC Immulite 2000 assay. The inter-assay coefficient variation was 4% at 28ng/l (6·2pmol/l). The same assay methodology was used in the healthy reference group.

Statistical Analysis

Pharmacokinetic (PK) parameters were computed by non-compartmental analysis. As only a 10mg dose of IR-HC was used, to assess relative bioavailability MR-HC was dose adjusted as follows: (AUC(MR-HC)×Dose(IR-HC)/(AUC(IR-HC)×Dose(MR-HC). Independent sample T-test was used to compare PK data of MR-HC and IR-HC. A significant difference was taken as $p < 0.05$. Dose proportionality was determined by comparing $AUC_{(0\text{-}inf)}$, and C_{max} of the 5mg, 15mg and 30mg MR-HC doses using a linear model approach on the log-transformed pharmacokinetic parameters versus log-transformed doses. The slope of the response against dose estimated, and dose proportionality accepted if the 90% confidence interval for the slope included unity.

In modeling we simulated giving MR-HC doses at different times and once or twice daily to establish whether MR-HC could produce a 24-hour physiological profile. To examine the best fit we calculated the ratio between the MR-HC AUC versus the physiological AUC at each of the pharmacokinetically relevant time intervals, e.g. the trapezoidal segment between each sampling interval. A ratio between 0·8 and 1·2 (within 20% of identity) was considered to indicate an acceptable MR-HC fit for that time interval. We could then determine the proportion of cortisol

levels after MR-HC that fell between the upper and lower 95% reference ranges for physiological cortisol levels.

Results

Defining the Physiological Cortisol Circadian Rhythm

A circadian rhythm is clearly demonstrated in the data from the healthy reference group (Figure1). A cosinor model with second harmonic gave an excellent fit to the mean cortisol data (r^2=0·97, p<0·001). All individuals had a significant (P<0.001) sinusoidal rhythm. Cortisol levels reached a peak at around 0832h (95%CI 0759h–0905h), then levels gradually decreased until reaching a nadir at 0018h (95%CI 2339 – 0058h). Two smaller peaks occurred at meal times. The means for all variables of the healthy reference group were similar to the previously published literature with overlap of all 95% CIs (Table 1) (20-29). In this cohort there was no difference in the level or phase of the circadian rhythm between men and women.

Pharmacokinetic Analysis of MR-HC and Comparison to IR-HC

Thirty two subjects were recruited. Four participants were withdrawn; two for violation of the protocol, one withdrew consent and one had an adverse event whilst on IR-HC (hiccups). The twenty eight other subjects completed the study (Table 3). 98% of all ACTH values were below the lower limit of the assay confirming dexamethasone-induced suppression of endogenous HPA axis, a mandatory precondition for correct pharmacokinetic analysis of cortisol bioavailability.

The cortisol concentration-time profiles following administration of MR-HC and IR-HC are shown in Figure 2. The summary statistics for the pharmacokinetic parameters is represented in Table 4 for comparison. The $mean_{geo}$ (90%CI) relative bioavailability of MR-HC formulations compared to that of IR-HC, as measured by dose-normalized AUC values, were: 100% (90-112%) for 5mg, 79% (66-95%) for 10mg, 86% (77-96%) for 15mg, and 69% (62-77%) for 30mg,

respectively. The corresponding analysis for the maximum exposure, as measured by C_{max} (without dose–normalization), indicated equivalence of the MR-HC 15mg dose to IR-HC 10mg [92% (77% - 107%)]. As expected from a delayed and sustained release formulation, the MR-HC 15mg dose showed a marked delay in mean T_{max}, prolongation in T_{lag}, and lower dose-normalized C_{max} compared to IR-HC 10mg dose [T_{max} (95% CI): (7.41 (6.16 - 8.66) hrs vs 1.8 (1.4 – 2.2) hrs, p≤0.001); T_{lag} (5.19 (4.3 – 6.09) hrs vs 0.5 (0.33 vs 0.67) hrs, p<0.001; dose-adjusted C_{max} (11.04 (9.02 – 13.06) mcg/dL vs 18.4 (16.9 – 19.9) mcg/dL, p≤0.001). All other formulations also showed characteristics of sustained and delayed release (Table 4). The peak concentrations and cortisol exposure increased predictably with increasing MR-HC dose. Dose proportionality was seen for MR-HC 5mg and 15mg: slope (90% CIs) were 0·82 (0·62-1·02) and 0·9 (0·69-1·1) for $AUC_{(0\text{-}inf)}$ and C_{max}, respectively. Dose proportionality was not shown between 15 mg and 30 mg MR-HC formulation: slopes (90%CI) for $AUC_{(0\text{-}inf)}$, and C_{max} were, 0·58 (0·30-0·85) and 0·64 (0·40-0·87), respectively. There was no correlation between weight and any variable of MR-HC pharmacokinetics.

Comparison of MR-HC to the Physiological Cortisol Profile

Administration of 30mg MR-HC provided the best cortisol exposure over 24 hours based on AUC compared to the physiological profile: mean (90%CI) = 88% (77–99%). However, the 30mg MR-HC C_{max} was above that seen for the physiological peak (mean±SEM = 24.9±1.1 vs 15.5±0.8 mcg/dL) and the peak occurred earlier than the physiological peak (mean: 0600h vs 0832h). The cortisol profile following MR-HC suggests that it only provides approximately 12 hours exposure to hydrocortisone (Figure 2) and when looking at the AUC for 12 hours after 2200h for 15mg MR-HC cortisol exposure was 84% (71–97%) of physiological, the C_{max} similar to physiological peak (16.6±1.4 vs 15.5±0.8 mcg/dL) but occurred earlier (0600h vs 0832h) when given at 2200h.

Modeling MR-HC Data to Provide Physiological Dosing Regimen

Based on the phase 1 MR-HC data obtained from the dose-response study using single dose regimes, we simulated giving MR-HC doses at different times and once or twice daily. For MR-HC 30mg given at 2200h, only 2 cortisol AUCs of the 16 (12.5%) fell within the 0.8 to 1.2 ratio. However, 12 (75%) of 16 cortisol AUCs fell within the 0.8 to 1.2 ratio for MR-HC given as 20mg at 2300h and 10mg at 0700 . Using this analysis we defined the best dose combination to provide physiological cortisol levels as either 15 or 20mg MR-HC at 2300h and 10mg MR-HC at 0700h. An example profile is shown in Figure 3 against physiological cortisol levels.

Discussion

Physiological cortisol replacement therapy for adrenal insufficiency is not possible with current oral hydrocortisone formulations (32). To address this we have developed a novel modified-release formulation of hydrocortisone (MR-HC). We have undertaken detailed pharmacokinetics of MR-HC and compared these with the physiological profile of cortisol in normal individuals. Based on pharmacokinetic modelling we suggest that a twice daily regimen of MR-HC given at 2300 and 0700h can provide levels of cortisol similar to normal physiological cortisol; AUC, C_{max} and T_{max}.

Defining the normal physiological profile of cortisol was critical to our study. We had access to a dataset of 33 healthy individuals who had undergone 20 minute blood sampling over a 24 hour period (19). The generalisability of these data is reflected by the fact that the peak and nadir cortisol, the timing of the peak and nadir, the duration of the quiescent phase and the onset of cortisol secretion and cosinor analysis were all very similar to that in the literature (20-29). The key variables in the cortisol profile that were defined were a peak cortisol of 15.5 mcg/dL (95%

reference range 11.7 – 20.6), with the acrophase at 0832h (95%CI 0759h – 0905h), a nadir cortisol of <2 mcg/dL (95% reference range 1.5 – 2.5) occurring at 0018h (95%CI 2339h – 0058h) and the quiescent phase (below the mesor) occurring from 1943h to 0531h.

We found no difference in physiological cortisol profiles according to weight or gender. Gender has previously been reported to have a minor effect on physiological cortisol levels (21) although not found in all studies (33). BMI does not have any significant effect on parameters quantifying the 24-hour cortisol profile in lean or modestly overweight individuals (21). In the literature there is an age effect on the cortisol profile and older subjects have an earlier onset of the cortisol rhythm (22). Most of our subjects were young and Caucasian. However, different populations and ethnic groups may exhibit differences in circadian cortisol rhythm parameters. For example, trough and peak cortisol levels occur earlier in Chinese men compared to Caucasians, possibly due to genetic differences and or environmental influences including social activity and everyday habits on entrainment of circadian rhythms, such as an average earlier bedtime (26).

The pharmacokinetics of MR-HC showed similar bioavailability to IR-HC with dose-proportionality for MR-HC between 5 and 15mg but not between 15 and 30mg. This is likely to be explained by the binding characteristics of CBG. Under basal conditions about 5% to 10% of circulating cortisol is free, about 75% is bound to CBG, and the remainder is bound to albumin. Increases in total plasma cortisol concentrations above 20 mcg/dL (550nmol/L) exceed the binding capacity of CBG and result in rapid increases in levels of free cortisol concentration, which thus exhibits more rapid clearance from the circulation (31). As the peak cortisol following 30mg MR-HC exceeded 20 mcg/dL, the lack of dose-proportionality is probably explained by this mechanism.

The profile following MR-HC was compared to physiological cortisol levels. The pharmacokinetic studies were all undertaken with subjects taking a single dose at 2200h. Based on this the onset of cortisol release preceded the end of the physiological quiescent phase and the peak preceded the timing of physiological peak cortisol. In addition a single dose of MR-HC could only replace the 24 hour AUC if the peak cortisol exceeded the physiological peak. We simulated, therefore, giving MR-HC at different time points and once or twice daily. In the simulations AUC at time intervals were compared with the normal circadian rhythm. This analysis shows that MR-HC 20mg and 10mg, given at 2300 and 0700h, respectively, could provide a cortisol profile with the least difference from normal physiological cortisol levels. However, this conclusion requires validation by an appropriate clinical study and it should be recognised that all simulations have been done on data derived from adults and a different regimen both in timing and dose will be required for children

The future of endocrine replacement lies in using modern pharmaceutical formulations to provide hormone replacement that replicates physiological hormone levels. We recognise that it is unlikely that any future drug regimen will be able to completely replicate the rapid adaptation of physiological cortisol secretion to different conditions of stress. However, we have shown that a modified release formulation of hydrocortisone that allows for delayed and sustained release of hydrocortisone can potentially replicate normal un-stressed physiological cortisol levels. Currently, in patients with adrenal insufficiency, health-related quality of life is significantly compromised, affecting their ability to work and cope with activities of daily life, and even more significant, mortality is increased. Future studies will determine the beneficial effects of physiological cortisol replacement but we have demonstrated here that it is possible to generate hydrocortisone formulations that provide cortisol profiles closer to baseline cortisol physiology.

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Table 1: Characteristics of Cortisol Circadian Rhythm – Comparison of Mean (±95% Confidence Intervals) Cortisol Concentrations, AUC and Time Variables in our healthy reference group (Darzy 2005) to similar variables in previously published data.

Author	Sample Mean Age (range)	AUC (h*mcg/dL)	Peak (mcg/dL)	Trough (mcg/dL)	24-hr Mean Cortisol (mcg/dL)	Time of Peak or Acrophase (hrs:min)	Time of Nadir (hrs:min)	Qui pha star (hrs
Darzy[1] (19) **(24 Males; 9 Females)**	27 (17-57)	160.2 (148.6 - 171.6)	16.0 (14.6 - 17.4)	2.5 (1.8 - 3.2)	6.7 (5.9 - 7.5)	08:32 (07:59 - 09:05)	00:18 (23:39 - 0:58)	19 (18 20
Other Authors[2] (20-29) **(127 Males; 38 Females)**	32 (19-59)	169.0 (138.5 - 207.0)	15.0 (11.6 - 19.0)	2.04 (1.0 - 3.2)	7.7 (5.7 - 15.8)	07:49 (06:28 - 09:01)	00:30 (22:00 - 02:00)	19 (16 22

[1] Variable Mean (95% Confidence Intervals)
[2] Values have been estimated from published data: Mean (Range)
[b1] Period starts when Cortisol level ~ 5.2 mcg/dL or less for at least 1h
[b2] Period starts when Cortisol level ~ 5.0 mcg/dL or less for at least 1h
[c1] Period starts when Cortisol levels ~ 5.2 mcg/dL or more for at least 1h
[c2] Period starts when Cortisol levels ~ 5.0 mcg/dL or more for at least 1h

Notes:

- Sample – subgroups used in analysis: (21) 20-29 yr subgroup, (22)19-25 yr subgroup, (24) S1 session, (25) Control group, (26) Chinese, 30 yr sub group
- AUC: Ref (23) - curve represents average of harmonic regressions
- Not used in analysis of: Trough: Ref (22); 24-hour mean cortisol: Ref (22, 28); Time of Nadir: Ref (26, 27, 29); Quiescent phase start: Ref (26, 27, 29); Quiescent phase end: Ref (27, 29)

Table 2: Study Scheme: Subjects admitted to Clinical Research Facility on day 1. Subjects tested on up to 3 occasions with a one week washout between treatments

STUDY ASSESSMENT	DAY 1		DAY 2			
	1800h	2200h	0600h	1200h	1800h	2200h
ACTH MEASUREMENT		ü	ü			
DEXAMETHASONE 1MG ORALLY	ü	ü	ü	ü	ü	
20* SUBJECTS DOSED WITH MR-HC 5MG, 15MG, 30MG OR IR-HC 10MG 12 SUBJECTS DOSED WITH MR-HC 10MG OR IR-HC 10MG		ü				
CORTISOL MEASUREMENT		Predose, postdose sampling every 30 minutes for the first 4 hours then at 5, 6, 7, 8, 10, 12, 15, 18 and 24 hours post dose				

*** 4 subjects withdrawn**

Table 3: Demographics for study subjects

Variable	Healthy Reference Group	Main Study Group
Number of Subjects	33	28
Sex	24 Males / 9 Females	28 Males
Mean Age (range)	27 (17 - 57)	33 (21 - 46)
Mean Weight (range)	67.1 (54 - 102)	80·0 (58 - 103)
Mean BMI (range)	22·9 (17 - 29)	25·6 (20 - 31)

Table 4: Pharmacokinetic Data for MR-HC and IR-HC

Variable Mean (SEM)	MR-HC 5mg (12 subjects)	MR-HC 10mg (12 subjects)	MR-HC 15mg (12 subjects)	MR-HC 30mg (12 subjects)	10mg IR Hydro-cortisone (24 subjects)
$AUC_{(0-24)}$ (hr*mcg/dL)	35.2 (4.0)	69.7 (6.2)	90.4 (9.0)	137.7 (10.5)	80.5 (4.2)
$AUC_{(0-inf)}$ (hr*mcg/dL)	41.5 (4.8)	77.3 (6.8)	98.5 (9.2)	143.2 (10.8)	88.2 (5.3)
C_{max} (mcg/dL)	6.4 (0.7)	10.9 (0.9)	16.6 (1.4)	24.9 (1.1)	18.4 (0.7)
T_{max} (hr)	8·25 (0·49)	7·83 (0·5)	7·41 (0·57)	7·17 (0·66)	1·8 (0·2)
T_{lag} (hr)	6·6 (0·47)	4·78 (0·45)	5·19 (0·43)	4·6 (0·44)	0·5 (0·08)
CL/F (L/hr)	13·8 (1·3)	14·08 (1·1)	16·56 (1·1)	22·08 (1·7)	11·04 (0·5)

$AUC_{(0-24)}$	Area under the plasma concentration-time curve for 24 hours
$AUC_{(0-inf)}$	Area under the plasma concentration-time curve extrapolated to infinity
C_{max}	Maximum observed concentration
T_{max}	Time to reach Cmax
T_{lag}	Time delay between drug administration and cortisol concentration > 3.5 mcg/dL
CL/f	Apparent clearance of drug after oral administration - CL/f=Dose/AUC(0-inf)

Figure Legends

Figure 1: Physiological Cortisol Circadian Rhythm: The figure shows the geometric mean (—) plus/minus 2SD (—) serum cortisol concentration calculated from 20-minute sampling over a 24-hour period in 33 healthy subjects. The fitted cosinor (—) is the average of harmonic regressions that were a fit for the individual subject data. Cortisol has a distinct circadian rhythm with a peak of 15.5 mcg/dL (95% reference range 11.7 – 20.6) occurring at 0832h and a nadir < 2.0 mcg/dL (95% reference range 1.5 – 2.5) at 0018h. Mesor = Midline estimating statistic of rhythm. Acrophase = Time of peak using a 24-hour clock with midnight taken as origin. Nadir = Time of trough cortisol level. The mean and 95%CI are shown for the mesor, acrophase and nadir.

Figure 2: Concentration – time profiles for MR-HC and IR-HC: Concentration – time profiles for different doses of MR-HC given at 2200h compared to 10mg IR-HC using geometric means (±SEM) of serum cortisol concentrations at 18 different time points over 24 hours. Profiles of MR-HC show a prolonged T_{max}, T_{lag}, and a lower dose-adjusted C_{max} (not shown) when compared to IR-HC, all typical of a formulation with delayed and sustained release characteristics.

Figure 3: Simulation of Physiological Cortisol rhythm using MR-HC: Phase 1 PK data were used to simulate giving MR-HC doses at different times and once or twice daily. PK modelling included comparison of MR-HC to the physiological cortisol rhythm over 12 and 24 hours, and at different time intervals (trapezoidal segments), using AUCs. The graph shows modeled concentration-time profile (—) obtained when giving 20mg (15mg + 5mg) MR-HC at 2300h and 10mg MR-HC at 0700h superimposed on the physiological cortisol rhythm (geometric mean (—) ± 2SD (—) serum cortisol concentration calculated from 20-minute sampling over a 24-hour period in 33 healthy subjects).