



This is a repository copy of *Developing oral chronotherapy for cortisol replacement in congenital adrenal hyperplasia*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/203846/>

Version: Published Version

Article:

Whitaker, M.J., Debono, M. and Ross, R.J. orcid.org/0000-0001-9222-9678 (2024)

Developing oral chronotherapy for cortisol replacement in congenital adrenal hyperplasia.

Clinical Endocrinology, 101 (4). pp. 311-317. ISSN 0300-0664

<https://doi.org/10.1111/cen.14976>

Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial (CC BY-NC) licence. This licence allows you to remix, tweak, and build upon this work non-commercially, and any new works must also acknowledge the authors and be non-commercial. You don't have to license any derivative works on the same terms. More information and the full terms of the licence here:

<https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>

Developing oral chronotherapy for cortisol replacement in congenital adrenal hyperplasia

Martin J. Whitaker¹ | Miguel Debono² | Richard J. Ross¹ 

¹Department of Oncology and Metabolism, University of Sheffield, Sheffield, UK

²Department of Endocrinology Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK

Correspondence

Richard J. Ross, University of Sheffield, Sheffield, UK.

Email: r.j.ross@sheffield.ac.uk

Abstract

The sun imposes a 24-h periodicity to life and circadian rhythms have evolved to maintain homeostasis through the day/night cycle. In humans, there is a central clock that controls the sleep/wake cycle which is paralleled metabolically by a fast/feed cycle. The clock maintains homeostasis by synchronising metabolism to the time of feeding. Loss of synchrony between the clock and hormonal rhythms results in loss of homeostasis as evidenced by obesity, depression, and diabetes in people undertaking shift work. Cortisol has a distinct circadian rhythm; peaking on waking and low at sleep onset. Loss of this rhythm in adrenal insufficiency is associated with a poor quality of life and increased mortality. To replace the cortisol rhythm requires chronotherapy and for this you need to define the key parameters of the target rhythm, create a formulation to replicate that rhythm, and then prove clinical benefit. The physiology of hormones is more complex than that of nonnative drugs. Hormones are secreted with varied rhythms, bound to multiple cognate binding proteins, and actively transported and cleared through enzymatic pathways in multiple organs. We have examined the diurnal rhythm of cortisol in healthy volunteers, created physiologically-based pharmacokinetic models, and tested various oral delayed and sustained formulations of hydrocortisone (development name, Chronocort) in clinical trials. The outcome from this work was the manufacture of modified-release hydrocortisone hard capsules (tradename Efmody, Diurnal Ltd), that replicate the cortisol diurnal rhythm and improve the disease control of congenital adrenal hyperplasia the commonest hereditary form of adrenal insufficiency.

KEYWORDS

adrenal insufficiency, chronotherapy, congenital Adrenal Hyperplasia

1 | RATIONALE FOR CHRONOTHERAPY

A central tenet in medicine is that disruption of homeostatic mechanisms leads to disease and effective therapy must re-establish normal physiology.¹ The sun imposes a 24-h periodicity to life on

earth that regulates much of human behaviour. Circadian rhythms have evolved in virtually all organisms to maintain homeostasis through the 24-h day/night cycle and these rhythms are controlled by clock genes.² In humans there is a central clock in the suprachiasmatic nucleus (SCN) that controls the diurnal sleep/wake

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2023 The Authors. *Clinical Endocrinology* published by John Wiley & Sons Ltd.

cycle which is paralleled metabolically by a fast/feed cycle. The clock maintains homeostasis by synchronising metabolism to the time of feeding; for example, regulating the hormones that maintain glucose homeostasis such as insulin and the glucocorticoid, cortisol. Loss of synchrony between the clock and these hormonal circadian rhythms results in loss of homeostasis as evidenced by obesity, depression, diabetes and insulin resistance in people undertaking shift work.^{3–5} In endocrine disorders such as diabetes and adrenal insufficiency, replicating the hormonal rhythms through chronotherapy is essential to restore normal physiology and maintain optimal health.

The importance of chronotherapy in improving disease outcomes is established. For example, insulin injections synchronised to feeding to optimally control diabetes mellitus and prevent long-term complications,⁶ and melatonin to treat insomnia.⁷ In patients with adrenal insufficiency, standard cortisol replacement therapy fails to replace the overnight rise in cortisol and is associated with fatigue, poor quality of life, and increased mortality.⁸ In congenital adrenal hyperplasia (CAH), the commonest congenital cause of adrenal insufficiency, the failure to replace the cortisol rhythm overnight results in poor disease control as the loss of cortisol negative feedback results in an excess adrenocorticotropin (ACTH) rise before waking that drives the production of excess adrenal androgens. Patients with CAH are commonly treated with supra-physiological doses of glucocorticoids to control the disease and the combination of poor disease control and excess glucocorticoid results in poor health outcomes including obesity, short stature, infertility and increased mortality.⁹ Thus, there is a need for chronotherapy to replace the diurnal rhythm of cortisol in CAH and to test whether it improves quality of life in adrenal insufficiency.

The challenge for chronotherapy in endocrinology has been formulating oral drugs to provide the appropriate hormonal rhythm. The physiology of hormones is more complex than that of nonnative drugs such as antibiotics. Hormones are secreted with specific but varied rhythms, frequently bound to multiple cognate binding proteins, and actively transported and cleared through various enzymatic pathways in multiple organs. To replicate a hormone's rhythm, you first need to understand its physiology and then devise a formulation that can replicate that physiology.

2 | THE PHYSIOLOGICAL CORTISOL RHYTHM

Cortisol has a distinct circadian rhythm first identified in the 1970s¹⁰ (Figure 1). Since then, several studies have mathematically defined the rhythm and summarised it numerically. The cortisol circadian rhythm has a period of ~24 h and can be described mathematically by a Fourier Series (cosinor model),¹¹ with a peak cortisol on waking and trough levels at sleep onset. The parameters of the cortisol circadian rhythm are very consistent across studies despite different cortisol assays and have been summarised in a manuscript by Debono et al.¹²; with the range of time for: peak 0630–0900 h, nadir 2200–0200 h, quiescent phase 1630–200 h and quiescent phase end 0300–0630 h.¹² Absolute levels for cortisol are also similar between studies and, using liquid chromatography with tandem mass spectrometry (LC-MS/MS) the gold standard assay, the geometric (10 and 90th centile) peak morning cortisol was 594 (409–973) nmol/L or 21.5 (14.8–35.3) µg/dL.^{12,13}

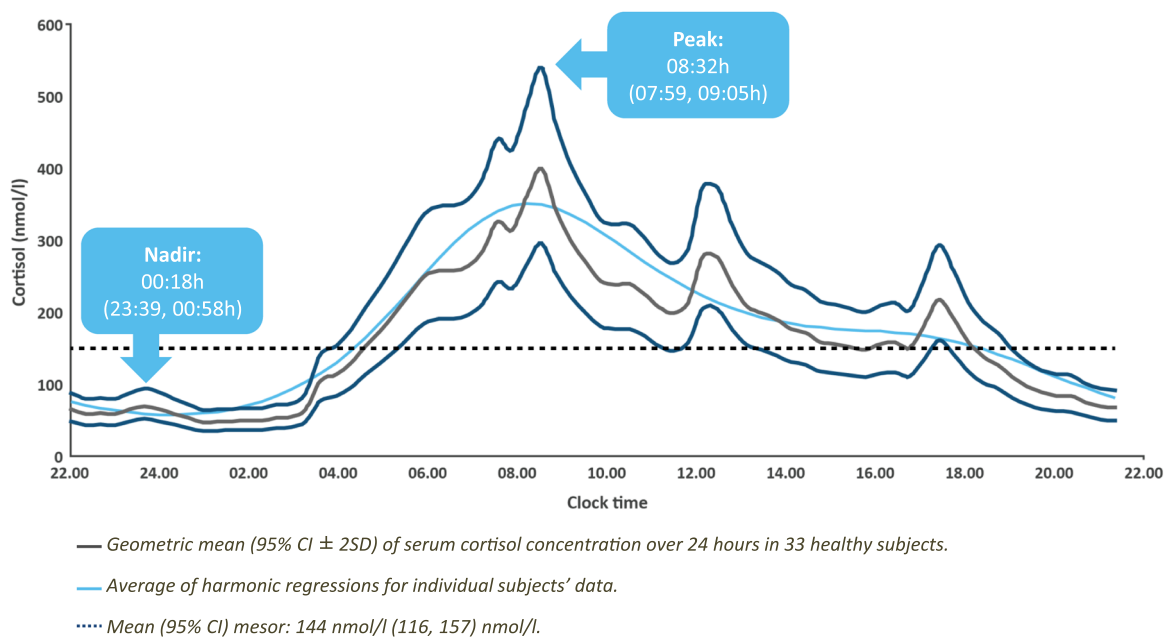


FIGURE 1 Cortisol circadian rhythm. Adapted from Debono et al.¹⁴ [Color figure can be viewed at wileyonlinelibrary.com]

The cortisol diurnal rhythm is determined by the central endogenous clock (pacemaker) of the hypothalamic-pituitary-adrenal axis, located in the hypothalamic supra-chiasmatic nucleus, which drives release of corticotropin releasing hormone, in turn leading to secretion of ACTH from the pituitary and thus cortisol from the adrenal. The central SCN clock has an approximate period length of 24.2 h and requires daily adjustment by photoperiod to synchronise to the 24-h day/night cycle. In addition to the central clock, there are molecular oscillators (peripheral clocks) in most mammalian cells.¹⁴ 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; however, glucocorticoids can phase delay or advance peripheral clock oscillators.¹⁵ Thus, cortisol maybe one of the secondary messengers from the central to peripheral clocks providing a strong argument for replacing the parameters of the cortisol diurnal rhythm in patients with adrenal insufficiency.

3 | PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELS OF HYDROCORTISONE

To design a hydrocortisone (pharmaceutical name for cortisol) formulation that will mimic the cortisol rhythm one needs to define the PK of hydrocortisone. To create a PBPK model it is important to consider the transport and clearance of cortisol in the circulation. In venous blood cortisol is 80% bound to cortisol binding globulin (CBG), 10% to albumin and approximately 10% is free (unbound fraction), the latter providing biological activity.¹⁶ As cortisol concentration exceeds ~550 nmol/L, CBG saturates so that the biologically active free cortisol increases. At these levels the clearance of total cortisol increases,¹⁷ and the disappearance rate is negatively correlated with CBG.¹⁸ A PBPK model was built, taking into account: hydrocortisone absorption parameters, plasma protein binding, ontogeny of CBG, metabolism of hydrocortisone and the ontogeny of the major metabolism pathways.¹⁹ The elimination of hydrocortisone/cortisol is complex; in many tissues, cortisol is converted to inactive cortisone by the enzyme 11 β -hydroxysteroid dehydrogenase 2 (11 β -HSD2) and the other major elimination enzyme is 5 α -reductase. There is a minor contribution from cytochrome P450 3A (CYP3A4) in the 6 β hydroxylation of cortisol.

Other metabolic pathways include 20 β -oxoreductase and 5 β -reductase.¹⁹ The PBPK model predicted immediate-release hydrocortisone pharmacokinetics (PK) in adults across the dose range 0.5 to 20 mg, with predicted/observed AUCs within 0.8–1.25-fold. The model also tightly predicted PK parameters for modified-release formulations, with AUCs within 0.8 to 1.25-fold after single and multiple dosing. Predicted modified-release formulation PK in 12–18-year olds showed PK to be similar to adults. The hydrocortisone PBPK model was an essential tool in formulation development to predict adult and paediatric PK of both immediate- and MRHC formulations and develop clinical dosing regimens.¹⁹ The PBPK model was generated using a 'bottom up' approach, where the model is built from the literature on absorption and metabolism, and based on the above maturation functions. In parallel work, a 'top down' approach, where the model is based on data derived from measurement of hydrocortisone PK, using clinical adult and paediatric data generated a nonlinear mixed-effects model,²⁰ and we then compared these two models in a 'middle-out' approach, where you combine information from both the bottom up and top down approach, with all three methodologies showing very similar parameters.²¹

PK studies require frequent sampling and this can be challenging especially in paediatric patient populations so the use of saliva to measure hydrocortisone PK was investigated. The enzyme 11 β -HSD2 is expressed in the salivary glands and rapidly inactivates cortisol by conversion to cortisone.²² In serum, levels of cortisone are approximately fourfold less than those of cortisol whereas in saliva levels of cortisone are approximately sixfold higher than those of cortisol and presumed to be generated from free serum cortisol during the production of saliva.²³ Thus, salivary cortisone predominantly reflects serum free cortisol and salivary cortisone reflects cortisol exposure under physiological conditions and after hydrocortisone administration.²⁴ Using the fact that salivary cortisone reflects free cortisol it was possible to confirm that oral hydrocortisone is completely absorbed and has ~100% bioavailability compared to intravenous hydrocortisone²⁵ (Table 1). Salivary cortisol profiles have been used to study hydrocortisone absorption in patients taking immediate release hydrocortisone and a modified release formulation called dual-release hydrocortisone.²⁶ An advantage of using salivary profiles and LC-MS/MS is that you can also measure other steroids in the same samples although validated assays are not widely available.

TABLE 1 Bioavailability of serum cortisol following administration of 20 mg of oral hydrocortisone.

| Route | Measure | AUC _{0-inf} (nmol/L.h) Mean \pm SD | C _{max} (nmol/L) Mean \pm SD | T _{max} (h) Median and Range | Bioavailability GeoMean and CI GeoMean |
|-------|----------------------|--|--|--|---|
| IV | Total serum cortisol | 2900 \pm 926 | 1629 \pm 491 | - | - |
| Oral | Total serum cortisol | 2779 \pm 1058 | 880 \pm 302 | 1.125 (0.5–1.5) | 1.0 (0.89–1.14) |
| IV | Salivary cortisone | 258 \pm 58 | 142 \pm 22 | - | - |
| Oral | Salivary cortisone | 239 \pm 48 | 88 \pm 17 | 1.0 (0.75–1.5) | 0.93 (0.83–1.05) |

Note: Adapted from Johnson et al.²⁵

4 | FORMULATION OF MODIFIED RELEASE HYDROCORTISONE (MRHC) CAPSULES

Having established the physiological rhythm of cortisol and the PK of hydrocortisone the challenge was to create a hydrocortisone formulation to replicate that rhythm. The PK of hydrocortisone mean that you need a modified release preparation if you wish to replicate the cortisol diurnal rhythm. Modelling suggested that you need a delayed and sustained release formulation of hydrocortisone (development programme name Chronocort), administered before bedtime, to replace the overnight rise in cortisol. The first formulation approach used an electrostatic deposition technology (Phoqus Pharmaceuticals plc) that could deposit a precise polymer coating on selective surfaces of a tablet formulation to enable the attainment of both delayed- and sustained-release functionality. Essentially a tablet was generated with sustained release hydrocortisone in the bottom layer and above an eroding layer with the sides and bottom of the tablet coated with an insoluble polymer coating. The tablet was therefore like a bucket with the eroding layer exposed to slowly erode as you passed through the gut and when the hydrocortisone layer was then exposed it would be released from the bottom of the bucket in a sustained fashion. The tablet worked; it gave the correct overnight cortisol profile in dexamethasone suppressed healthy volunteers but with reduced bioavailability²⁷; and in a phase 2 study the tablet improved disease control in patients with CAH.²⁸ However, the tablet did not have a reproducible dissolution profile and it was challenging to scale up the technology. So the first formulation, a Chronocort tablet, showed it was possible to replicate the night-time cortisol rise and improve CAH disease control but the technology ultimately failed.²⁹

An alternative approach to the development of a modified release formulation of hydrocortisone is an oral tablet consisting of an immediate-release coating covering a sustained release core (IRSR) (tradename Plenadren).³⁰ It is designed to be given once daily in the morning and provide daytime levels of cortisol but does not reproduce the overnight diurnal cortisol rhythm. The IRSR formulation, like the first Chronocort formulation, has reduced bioavailability by about 20%. Plenadren was approved for the treatment of adrenal insufficiency in Europe in 2011.³¹

The subsequent formulation approach to generating Chronocort used a conventional modified-release technology platform; multiparticulates using drug and polymer-layering, to integrate the delayed- and sustained-release features into a revised circadian formulation of hydrocortisone.²⁹ The key advantage of the multiparticulate technology is that with judicious formulation design, the delayed release functionality can be controlled independently from the sustained release functionality thereby providing scope to optimise the PK profile of hydrocortisone against the circadian pattern of cortisol. Additionally, using multi-particulates allows you to generate a wide dose range which is required when titrating treatment in patients with adrenal insufficiency. Six different formulations were generated (Table 2), with varying degrees of delayed and sustained release including a control with no sustained release. All the formulations with sustained release showed reduced bioavailability in dexamethasone suppressed healthy volunteers and it was thought this was because the formulation moved into the colon whilst still releasing its load. In contrast, the 006 formulation, with only a delayed release coating, showed both delayed release and sustained absorption with good target bioavailability³² (Figure 2). This 006 Chronocort formulation, is a multiparticulate with a polymer coat designed to dissolve at a pH of >6.8 which is what pH rises above in the last third of the small bowel. So, the assumption is that this formulation worked because when you take it last thing at night, a time at which the

TABLE 2 MRHC formulations with varying delayed and sustained release coatings.

| Formulation ID | Sustained release coat: thickness as % of drug core | Enteric coat: pH at which mean dissolution occurs |
|----------------|---|---|
| DIURF-000 | 25.0 | 6.8 |
| DIURF-001 | 20.0 | 6.5 |
| DIURF-002 | 12.5 | 6.5 |
| DIURF-003 | 12.5 | 6.0 |
| DIURF-004 | 10.0 | 6.8 |
| DIURF-005 | 8.0 | 6.8 |
| DIURF-006 | None | 6.8 |

Abbreviation: MRHC, modified-release hydrocortisone.

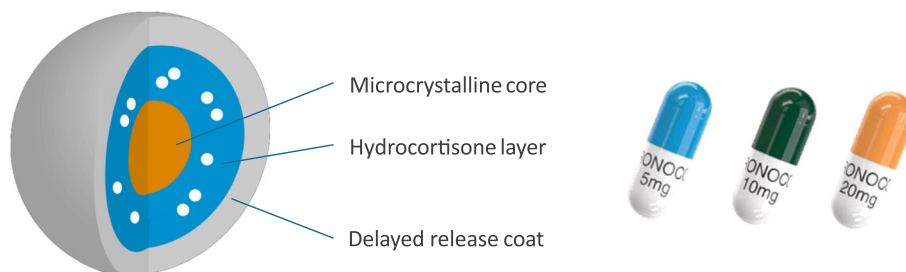


FIGURE 2 Cross-sectional diagram of multilayered, multiparticulate Chronocort formulation (DIURF 006) which is contained in capsules with the number of granules made up to deliver 5, 10 and 20 mg hydrocortisone. [Color figure can be viewed at wileyonlinelibrary.com]

bowel slows down, this increases the delay to release and in the last third of the small bowel there is reduced movement, and in view of the presence of water at the absorptive surface (hydrocortisone is very insoluble in water) you get sustained absorption. Given twice daily, last thing at night and first thing in the morning the 006 Chronocort formulation reproduced the cortisol diurnal rhythm and physiological levels of cortisol exposure in dexamethasone suppressed healthy volunteers.³² Key learnings in the formulation programme were that sustained release resulted in reduced bioavailability and that you require twice daily dosing to replicate the cortisol diurnal rhythm.

5 | CLINICAL TRIALS WITH MRHC

Designing clinical trials in drug development is complex as the outputs need to satisfy several different parties; not least being the regulators and health economists. Design is particularly challenging when you are developing a drug for a disease such as adrenal insufficiency and CAH where there have been no previous studies defining biomarkers and clinical outcomes. In adrenal insufficiency there are no biomarkers to monitor glucocorticoid replacement and the main complaint of patients is fatigue and impaired quality of life. CAH guidelines recommend using the lowest glucocorticoid dose to control adrenal androgen production and the biomarkers of disease control are the adrenal hormones 17-hydroxy-progesterone (17OHP) and androstenedione (A4).³³ The availability of biomarkers in CAH made it the initial target disease to treat with chronotherapy. A phase 2 switch study was undertaken in 16 adults (eight women) with classic CAH. Patients were switched from their standard glucocorticoid therapy to MRHC hard capsules and followed for 6 months.³⁴ The cortisol profile on MRHC in the CAH patients was similar to physiological cortisol levels, the dose of hydrocortisone was

less on MRHC, and the 17OHP and A4 levels were lower compared to standard glucocorticoid therapy with the majority of patients 0900 h 17OHP moving into within three times the upper limit of normal a previously defined optimal range³⁵ (Figure 3).

Based on the encouraging results from the Phase 2 study a multicentre Phase 3 parallel arm 6-month study of MRHC versus standard therapy follow by an open label MRHC extension safety study³⁶ was performed. The Phase 3 study showed that MRHC improved disease control of CAH in lowering the 17OHP first thing in the morning and throughout the day, whereas on standard treatment 17OHP rose to high levels in the morning (Figure 4). The study missed its primary endpoint because this was based on a log-transformed mean of the 24-h data which obscured the beneficial morning suppression of 17OHP at 6 months. The Phase 3 extension study demonstrated that the improved control of CAH, as measured

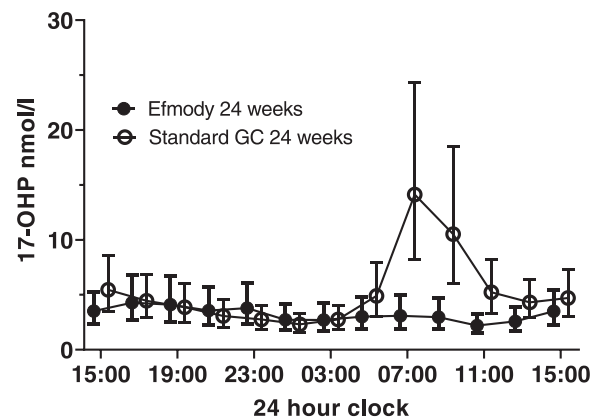


FIGURE 4 24 h 17OHP profile after randomisation to either titrated standard treatment or Efmody showing that Efmody controls the overnight rise in 17OHP. 17OHP, 17-hydroxy-progesterone.

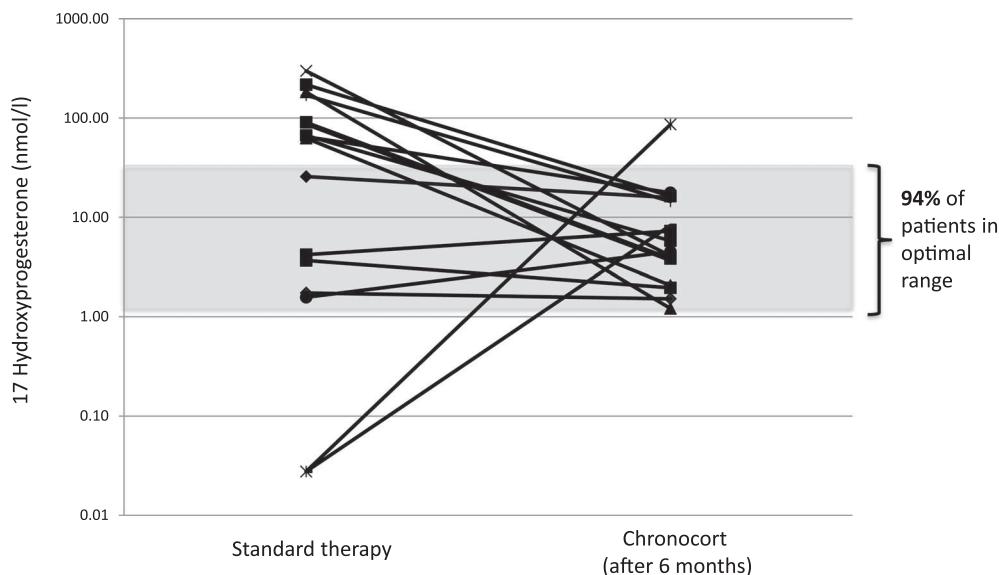


FIGURE 3 0900 h 17OHP in 16 patients with classic CAH on their standard treatment and after 6 months treatment with Chronocort. 17OHP, 17-hydroxy-progesterone; CAH, congenital adrenal hyperplasia.

by lower 17OHP levels, was maintained on a lower replacement dose of glucocorticoid, median daily dose 20 mg, and furthermore, patient-reported clinical benefit was shown with improved fertility followed by pregnancy in 6 women with CAH and 4 female partners of men with CAH.³⁶ Regarding safety there were 3 adrenal crises in the standard treatment group and none on MRHC in the 6-month randomised study and in the extension study on MRHC the incidence of adrenal crisis was at the low end of that reported in cohort studies.³⁶ One of the key learnings from the MRHC studies was that when 17OHP levels were controlled through the 24 h the A4 levels were low showing that A4 can be used to monitor for under-treatment with glucocorticoid but not for over treatment.³⁷ The data from the clinical development plan for MRHC was submitted to the EMA and received marketing authorisation approval from the European Commission in 2021. A randomised double-blind study of MRHC versus hydrocortisone is currently underway in patients with CAH and a similar study is also recruiting in patients with primary adrenal insufficiency.

6 | CONCLUSIONS

A major learning as part of this programme was the length of time it takes to develop a new therapy and the amount of money that must be raised to take a new therapy to market. Diurnal, the University of Sheffield spinout company, raised ~£100 million over its ~20-year life to cover the development of MRHC, its production, the clinical trials, regulatory submissions and reimbursement discussions. Defining the unmet need at the start of the programme was essential when it came to drug and study design. Formulating a hormone to deliver a physiological rhythm is challenging because absorption and clearance are not always predictable, and one is limited by gut physiology. Finally, defining and demonstrating outcomes in orphan diseases is hard and one needs to be careful moving from Phase 2 to Phase 3 studies when defining primary outcomes. Despite these challenges in its development Efmody is bringing clinical benefit to patients with CAH including improved disease control on a lower glucocorticoid dose with evidence of improved fertility.

CONFLICT OF INTEREST STATEMENT

R. J. R. and M. J. W. were Directors of Diurnal Ltd. The remaining author declares no conflict of interest.

ORCID

Richard J. Ross  <http://orcid.org/0000-0001-9222-9678>

REFERENCES

1. Billman GE. Homeostasis: the underappreciated and far too often ignored central organizing principle of physiology. *Front Physiol.* 2020;11:200.
2. Kim YH, Lazar MA. Transcriptional control of circadian rhythms and metabolism: a matter of time and space. *Endocr Rev.* 2020;41:707-732.
3. Oosterman JE, Wopereis S, Kalsbeek A. The circadian clock, shift work, and tissue-specific insulin resistance. *Endocrinology.* 2020;161(12):1-11.
4. Morris CJ, Aeschbach D, Scheer FAJL. Circadian system, sleep and endocrinology. *Mol Cell Endocrinol.* 2012;349:91-104.
5. Morris CJ, Yang JN, Scheer F. The impact of the circadian timing system on cardiovascular and metabolic function. *Prog Brain Res.* 2012;199:337-358.
6. Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: diabetes control and complications trial. diabetes control and complications trial research group. *J Pediatr.* 1994;125:177-188.
7. Lemoine P, Nir T, Laudon M, Zisapel N. Prolonged-release melatonin improves sleep quality and morning alertness in insomnia patients aged 55 years and older and has no withdrawal effects. *J Sleep Res.* 2007;16:372-380.
8. Ngaosuwan K, Johnston DG, Godsland IF, et al. Increased mortality risk in patients with primary and secondary adrenal insufficiency. *J Clin Endocrinol Metabol.* 2021;106:e2759-e2768.
9. Merke DP, Auchus RJ. Congenital adrenal hyperplasia due to 21-Hydroxylase deficiency. *N Engl J Med.* 2020;383:1248-1261.
10. Krieger DT, Allen W, Rizzo F, Krieger HP. Characterization of the normal temporal pattern of plasma corticosteroid levels. *J Clin Endocrinol Metab.* 1971;32:266-284.
11. Chakraborty A, Krzyzanski W, Jusko WJ. Mathematical modeling of circadian cortisol concentrations using indirect response models: comparison of several methods. *J Pharmacokinet Biopharm.* 1999;27:23-43.
12. Debono M, Ghobadi C, Rostami-Hodjegan A, et al. Modified-release hydrocortisone to provide circadian cortisol profiles. *J Clin Endocrinol Metabol.* 2009;94:1548-1554.
13. Whitaker MJ, Debono M, Huatan H, Merke DP, Arlt W, Ross RJ. An oral multiparticulate, modified-release, hydrocortisone replacement therapy that provides physiological cortisol exposure. *Clin Endocrinol.* 2013;80:554-561.
14. Dunlap JC. Molecular bases for circadian clocks. *Cell.* 1999;96:271-290.
15. Balsalobre A, Brown SA, Marcacci L, et al. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science.* 2000;289:2344-2347.
16. Baxter JD, Forsham PH. Tissue effects of glucocorticoids. *Am J Med.* 1972;53:573-589.
17. Tunn S, Möllmann H, Barth J, Derendorf H, Krieg M. Simultaneous measurement of cortisol in serum and saliva after different forms of cortisol administration. *Clin Chem.* 1992;38:1491-1494.
18. Bright GM, Bright GM. Corticosteroid-binding globulin influences kinetic parameters of plasma cortisol transport and clearance. *J Clin Endocrinol Metabol.* 1995;80:770-775.
19. Bonner JJ, Burt H, Johnson TN, Whitaker MJ, Porter J, Ross RJ. Development and verification of an endogenous PBPK model to inform hydrocortisone replacement dosing in children and adults with cortisol deficiency. *Eur J Pharm Sci.* 2021;165:105913.
20. Melin J, Parra-Guillen ZP, Hartung N, et al. Predicting cortisol exposure from paediatric hydrocortisone formulation using a Semi-Mechanistic pharmacokinetic model established in healthy adults. *Clin Pharmacokinet.* 2017;57:515-527.
21. Michelet R, Bindellini D, Melin J, et al. Insights in the maturational processes influencing hydrocortisone pharmacokinetics in congenital adrenal hyperplasia patients using a middle-out approach. *Front Pharmacol.* 2023;13:1090554.
22. Smith RE, Maguire JA, Stein-Oakley AN, et al. Localization of 11 beta-hydroxysteroid dehydrogenase type II in human epithelial tissues. *J Clin Endocrinol Metab.* 1996;81:3244-3248.

23. Perogamvros I, Owen LJ, Newell-Price J, Ray DW, Trainer PJ, Keevil BG. Simultaneous measurement of cortisol and cortisone in human saliva using liquid chromatography-tandem mass spectrometry: application in basal and stimulated conditions. *J Chromat B*. 2009;877:3771-3775.
24. Debono M, Harrison RF, Whitaker MJ, et al. Salivary cortisone reflects cortisol exposure under physiological conditions and after hydrocortisone. *J Clin Endocrinol Metabol*. 2016;101:1469-1477.
25. Johnson TN, Whitaker MJ, Keevil B, Ross RJ. Bioavailability of oral hydrocortisone corrected for binding proteins and measured by LC-MS/MS using serum cortisol and salivary cortisone. *J Bioequival Bioavailabil*. 2018;10:1-3.
26. Ceccato F, Selmin E, Sabbadin C, et al. Improved salivary cortisol rhythm with dual-release hydrocortisone. *Endocr Connect*. 2018;7:965-974.
27. Newell-Price J, Whiteman M, Rostami-Hodjegan A, et al. Modified-release hydrocortisone for circadian therapy: a proof-of-principle study in dexamethasone-suppressed normal volunteers. *Clin Endocrinol*. 2008;68:130-135.
28. Verma S, Vanryzin C, Sinaii N, et al. A pharmacokinetic and pharmacodynamic study of delayed- and extended-release hydrocortisone (Chronocort™) vs. conventional hydrocortisone (Cortef™) in the treatment of congenital adrenal hyperplasia. *Clin Endocrinol*. 2010;72:441-447.
29. Whitaker MJ, Huatan H, Ross RJ. Chronotherapy based on modified-release hydrocortisone to restore the physiological cortisol diurnal rhythm. *Drug Delivery Transl Res*. 2023;13:1-8.
30. Johannsson G, Bergthorsdottir R, Nilsson AG, Lennernas H, Hedner T, Skrtic S. Improving glucocorticoid replacement therapy using a novel modified-release hydrocortisone tablet: a pharmacokinetic study. *Eur J Endocrinol*. 2009;161:119-130.
31. Johannsson G, Nilsson AG, Bergthorsdottir R, et al. Improved cortisol exposure-time profile and outcome in patients with adrenal insufficiency: a prospective randomized trial of a novel hydrocortisone dual-release formulation. *J Clin Endocrinol Metabol*. 2012;97:473-481.
32. Whitaker MJ, Debono M, Huatan H, Merke DP, Arlt W, Ross RJ. An oral multiparticulate, modified-release, hydrocortisone replacement therapy that provides physiological cortisol exposure. *Clin Endocrinol*. 2014;80:554-561.
33. Speiser PW, Arlt W, Auchus RJ, et al. Congenital adrenal hyperplasia due to steroid 21-Hydroxylase deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metabol*. 2018;103:4043-4088.
34. Mallappa A, Sinaii N, Kumar P, et al. A phase 2 study of chronocort, a modified-release formulation of hydrocortisone, in the treatment of adults with classic congenital adrenal hyperplasia. *J Clin Endocrinol Metabol*. 2015;100:1137-1145.
35. Arlt W, Willis DS, Wild SH, et al. Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. *J Clin Endocrinol Metab*. 2010;95:5110-5121.
36. Merke DP, Mallappa A, Arlt W, et al. Modified-Release hydrocortisone in congenital adrenal hyperplasia. *J Clin Endocrinol Metabol*. 2021;106:e2063-e2077.
37. Prete A, Auchus RJ, Ross RJ. Clinical advances in the pharmacotherapy of congenital adrenal hyperplasia. *Eur J Endocrinol*. 2022;186:R1-R14.

How to cite this article: Whitaker MJ, Debono M, Ross RJ. Developing oral chronotherapy for cortisol replacement in congenital adrenal hyperplasia. *Clin Endocrinol*. 2023;1-7. doi:10.1111/cen.14976