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Original Research

Modified-release hydrocortisone is associated with lower plasma renin activity in patients with salt-wasting congenital adrenal hyperplasia

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Abstract

Objective: Poorly controlled salt-wasting (SW) congenital adrenal hyperplasia (CAH) patients often require high 9α-fluorocortisol doses as they show high levels of 17-hydroxyprogesterone (17OHP), which is a mineralocorticoid (MC)-receptor antagonist.

Design: We investigated the renin–angiotensin–aldosterone system in patients with SW-CAH receiving twice daily modified-release hydrocortisone (MR-HC, Efmody) compared with standard glucocorticoid (GC) therapy.

Methods: Data were analyzed from the 6-month, phase 3 study of MR-HC ($n = 42$) versus standard GC therapy ($n = 41$). MC replacement therapy remained unchanged throughout the study. Blood pressure, serum potassium, serum sodium, plasma renin activity (PRA), and serum 17OHP and androstenedione concentrations were analyzed at baseline, 4, 12, and 24 weeks.

Results: The median serum 17OHP in the morning was significantly lower on MR-HC compared with standard GC at 24 weeks (2.5 nmol L^{-1} (IQR 8.3) versus 10.5 nmol L^{-1} (IQR 55.2), $P = .001$). PRA decreased significantly from baseline to 24 weeks in patients on MR-HC (0.83 ng L^{-1} s^{-1} (IQR 1.0) to 0.48 ng L^{-1} s^{-1} (IQR 0.61), $P = .012$) but not in patients on standard GC (0.53 ng L^{-1} s^{-1} (IQR 0.66) to 0.52 ng L^{-1} s^{-1} (IQR 0.78), $P = .613$). Serum sodium concentrations increased from baseline to 24 weeks in patients on MR-HC (138.8 ± 1.9 mmol L^{-1} to 139.3 ± 1.8 mmol L^{-1}, $P = .047$), but remained unchanged on standard GC (139.8 ± 1.6 mmol L^{-1} to 139.3 ± 1.9 mmol L^{-1}, $P = .135$). No significant changes were seen in systolic and diastolic blood pressure and serum potassium levels.

Conclusion: 6 months of MR-HC therapy decreased PRA and increased sodium levels indicating a greater agonist action of the 9α-fluorocortisol dose, which may be due to the decreased levels of the MC-receptor antagonist 17OHP.

Keywords: plasma renin activity, aldosterone, hydrocortisone, fludrocortisone, prednisolone, dexamethasone, adrenal insufficiency, 21-hydroxylase deficiency

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Significance

This is the first study to investigate the renin–angiotensin–aldosterone system in patients with salt-wasting congenital adrenal hyperplasia (SW-CAH) receiving twice daily modified-release hydrocortisone (MR-HC, Efmody) compared with standard glucocorticoid medication. 6 months of MR-HC significantly decreased plasma renin activity and significantly increased sodium levels in SW-CAH patients without significant change in glucocorticoid or mineralocorticoid dose. MR-HC also led to improved biochemical control with median 17-hydroxyprogsterone (17OHP) levels below 10 nmol L^{-1}. Since 17OHP is a known mineralocorticoid receptor antagonist, the observed greater agonist action of the mineralocorticoid substitution may be due to lower levels of 17OHP. More effective and lower-dose mineralocorticoid substitution with MR-HC treatment could lead to clinical benefits in patients with SW-CAH and reduce long-term consequences risk.

Introduction

Patients with salt-wasting congenital adrenal hyperplasia (SW-CAH) due to classic 21-hydroxylase deficiency (21-OHD) require glucocorticoid (GC) and mineralocorticoid (MC) replacement therapy.

The GC replacement therapy aims first at delivering the daily physiological amount of cortisol to the patient, and second to normalize the increased precursor steroids, mainly 17alpha-hydroxyprogesterone (17OHP), and preventing their conversion to androgens.[1]

In recent years, the importance of preserving the physiologic circadian rhythm of cortisol became the focus of research on novel approaches to glucocorticoid therapy. Immediate-release preparations of hydrocortisone and longer-acting glucocorticoid preparations fail to mimic the early-morning (3-4 a.m.) cortisol surge when given at the usual wake-up times, which means that excess ACTH stimulation of the adrenal often remains unopposed and results in increased steroid precursor and subsequently adrenal-derived androgen concentrations. A novel, oral modified-release hydrocortisone preparation (MR-HC, Efmody, Diurnal Ltd) has been shown to better mimic the normal circadian rhythm of cortisol,[2] resulting in improved biochemical control as compared to standard GC therapy in CAH.[3]

MC replacement therapy is often considered simple and straightforward and has not been altered since the introduction of 9α-fluorocortisol (fludrocortisone) over 60 years ago.[4] For MC replacement, 9α-fluorocortisol is recommended usually as a single morning dose of 0.05-0.2 mg, although a twice daily regimen has been observed to be more effective.[5] Relative 9α-fluorocortisol doses vary widely among the different age subgroups of SW-CAH, with the highest doses administered in the under-1-year-old and 1- to 8-year-old groups and a relative decrease in dose with older age.[6] MC replacement therapy is monitored by clinical assessment such as blood pressure and biochemical markers, eg, serum sodium and potassium levels as well as plasma renin activity (PRA) or plasma renin concentration.[7,8] The relationship of PRA with MC replacement is complex because there is little standardization in the method of collecting PRA with posture, timing, and adherence complicating the previous cohort studies.[9]

Poorly controlled SW-CAH patients often require higher daily doses of 9α-fluorocortisol than patients with autoimmune primary adrenal insufficiency.[10] It has been suggested that progesterone and its metabolites exacerbate MC deficiency in SW-CAH patients through antagonism at the human mineralocorticoid receptor (hMR),[11,12] and it has been demonstrated *in vitro*[13] and *in vivo*[14] that 17OHP is a potent hMR antagonist, which might explain the need for increased 9α-fluorocortisol requirements in poorly controlled SW-CAH.

The aim of this study was to investigate the renin–angiotensin–aldosterone (RAA) system in a carefully controlled study with standardized collection of PRA. We tested the hypothesis that better control of 17OHP on MR-HC would improve the efficacy of MC replacement therapy.

Subjects and methods

Study design

Data were collected as part of the phase 3 DIUR-005 efficacy trial of the MR-HC Efmody in patients with classic 21-OHD CAH (for details see Merke *et al.*[3]). The study protocols for the phase 3 extension study were approved by local ethics/institutional review boards and the Medicines and Healthcare Products Regulatory Agency (NCT03062280, Eudract 2015-005448-32) (see Merke *et al.*[3]). Written consent has been obtained from each patient or subject after a full explanation of the purpose and nature of all procedures used. The trials were performed in accordance with the principles of the Declaration of Helsinki.

Clinical or biochemical evidence of renal or liver disease led to study exclusion. Co-medication that was considered necessary for the patients' health status and well-being could be continued during the course of the study. However, this excluded co-medication which had to be administered on a daily basis and was known to interfere with glucocorticoid metabolism.

All patients at baseline were on a stable GC dose for the previous 6 months and received sufficient MC replacement therapy with a PRA <1.5 times the upper limit.

122 patients were randomized to either MR-HC or their standard GC medication throughout the course of the study. Medication at the time of study entry was divided into three subgroups: (1) hydrocortisone (HC) alone; (2) prednisone or prednisolone, alone or in combination with hydrocortisone; (3) dexamethasone, alone or in combination with another GC. Standard GC dose was documented in hydrocortisone dose equivalent, calculated as prednisone dose multiplied by 5 and dexamethasone dose multiplied by 80.[15] Patients randomized to MR-HC received the initial dose corresponding to the hydrocortisone dose equivalent to their baseline therapy, with approximately one-third of the daily dose taken at 07:00 hours and two-thirds of the daily dose taken at 23:00 hours. Patients were assessed at baseline, weeks 4, 12, and 24. GC dose titration was performed at weeks 4 and 12 in both groups according to the same rules, with the decision

regarding changing the dose made by two independent physicians blinded to treatment.

Eighty-eight patients were diagnosed as having SW-CAH based on mineralocorticoid replacement therapy and genetic mutation status documented in the medical history. Patients with MC replacement therapy but documented simple virilizing CAH according to their genetic mutation status were excluded from this analysis.

Outcome measures in our study concerning MC control were PRA, serum sodium and potassium concentrations, systolic and diastolic blood pressure, serum 17OHP and androstenedione (A4) concentration at baseline, weeks 4, 12, and 24, as well as GC and MC doses at baseline and week 24. Alcohol and food could only be consumed until 21:00 hours prior to each study visit. All blood samples were taken in the morning between 07:00 and 09:00 hour, PRA values were collected after the patients had been in the supine position for 30 minutes and before taking the first morning dose of glucocorticoid or mineralocorticoid. Steroid hormones and PRA were measured using high-performance liquid chromatography-tandem mass spectrometry with a reference range for PRA of 0.007-1.62 ng L^{-1} s^{-1}. Blood pressure was measured once in the morning.

The standard GC group was subdivided into patients staying on HC, predniso(lo)ne or dexamethasone only, or receiving a combination of GC preparations. Further sub-analyses were performed after excluding two patients who received antihypertensive medication and two patients who received a drospirenone-containing contraceptive (due to its known anti-MC effect).[16]

Statistical analysis

All variables were tested for normal distribution using the Shapiro-Wilk test. For correlation analysis Spearman's rho was used, as most variables did not meet the criteria of normal distribution. The data analysis consisted of two parts, one assessing the within-subject design and the other assessing the between-subject design. For this purpose, groups were analyzed as MR-HC versus standard GC, as well as MR-HC versus HC versus predniso(lo)ne/dexamethasone. Since antihypertensive medication, drospirenone-containing contraceptives and the menstrual cycle interfere with or influence the RAA system, additional sub-analyses were carried out. To determine differences in the within-subject design (differences within one group between several time points), a dependent sample *t*-test was performed for normally distributed data, or for non-normally distributed data the Wilcoxon test and Friedman test were performed. To determine differences in the between-study design (differences between groups at one time point), a Mann–Whitney *U* test and Kruskal–Wallis test with Bonferroni-holm correction for multiple testing were performed. When data from all time points met the criteria of normally distributed data, an additional ANCOVA for repeated-measures was performed. Linear regression was carried out to identify significant predictors for the outcome variables representing the RAA system with significant differences between groups or time points.

Descriptive statistics for normally distributed data is given as mean (M) and standard deviation (SD), and for non-normally distributed data as median (Md) and interquartile range (IQR). Missing values in outcome variables at baseline or week 24 led to exclusion. Otherwise, missing values were replaced by mean values of the respective variables. A *P*-value of <.05 was considered significant. For statistical analysis IBM SPSS Statistics 26.0 was used.

Results

Patient characteristics

Eighty-eight SW-CAH patients completed the 6-month trial, five were excluded from statistical analysis due to missing values. Therefore, 83 patients were included in the analysis. The median age of the patients was 35 (19-66) years, 29 were men (34.9%) and all but one patient were Caucasian. Forty-two patients (50.6%) were randomized to MR-HC and 41 (49.4%) to continue on their standard GC. Of the 41 patients continuing on their standard GC therapy, 23 (56.1%) were on HC, 16 (39.0%) on predniso(lo)ne alone or in combination with HC, and 2 (4.9%) were on dexamethasone alone or in combination with another GC (see Figure 1). The median age of the MR-HC group was 33 (19-50) years, the median age of the standard GC groups was 37 (19-66) years. There was no statistically significant age difference between the two patient groups.

9α-fluorocortisol was used for MC therapy in all patients and its dose was changed in only three patients during the course of the study. In two patients, the MC dose was changed only temporarily and by the end of the study (week 24) they were back to their original MC baseline dose. Only one patient had a permanent change in 9α-fluorocortisol dose during the course of the study from 0.2 to 0.25 mg d^{-1}, thus, 9α-fluorocortisol dose was considered stable over the course of the study. An overview of the baseline characteristics of treatment groups is given in Table 1.

Correlation of PRA with electrolytes and steroids

At baseline, PRA in the entire study population correlated negatively with serum sodium concentration ($r = -0.44$; $P < .001$) and positively with serum potassium ($r = 0.44$; $P < .001$) and serum 17OHP ($r = 0.27$; $P = .014$). At week 24, PRA correlated negatively with serum sodium ($r = -0.42$; $P < .001$) and positively with serum potassium ($r = 0.57$; $P < .001$), but not with serum 17OHP ($r = 0.19$; $P = .088$).

Divided by group, at week 24, PRA correlated negatively with serum sodium ($r = -0.32$; $P = .039$) and positively with serum potassium ($r = 0.60$; $P < .001$) in patients receiving MR-HC. In patients staying on standard GC therapy, PRA correlated negatively with serum sodium ($r = -0.49$; $P = .001$) and positively with serum potassium ($r = 0.53$; $P < .001$). An overview of the correlation analysis is depicted in Tables 2 and 3.

Differences between groups at baseline and week 24

At baseline, serum sodium in the patients staying on standard GC was significantly higher than in patients receiving MR-HC (139.8 mmol L^{-1} (SD 1.6) versus 138.8 mmol L^{-1} (SD 1.9); $P = .020$). The analysis of the subgroups also revealed that patients staying on predniso(lo)ne/dexamethasone had a significantly higher median 9α-fluorocortisol dose at baseline than patients staying on HC (0.10 mg d^{-1} (IQR 0.10) versus 0.075 (IQR 0.050); $P = .048$). Patients staying on predniso(lo)ne/dexamethasone received a significantly higher median hydrocortisone dose equivalent at baseline compared to patients staying on HC (30.0 mg d^{-1} (IQR 16.3) versus

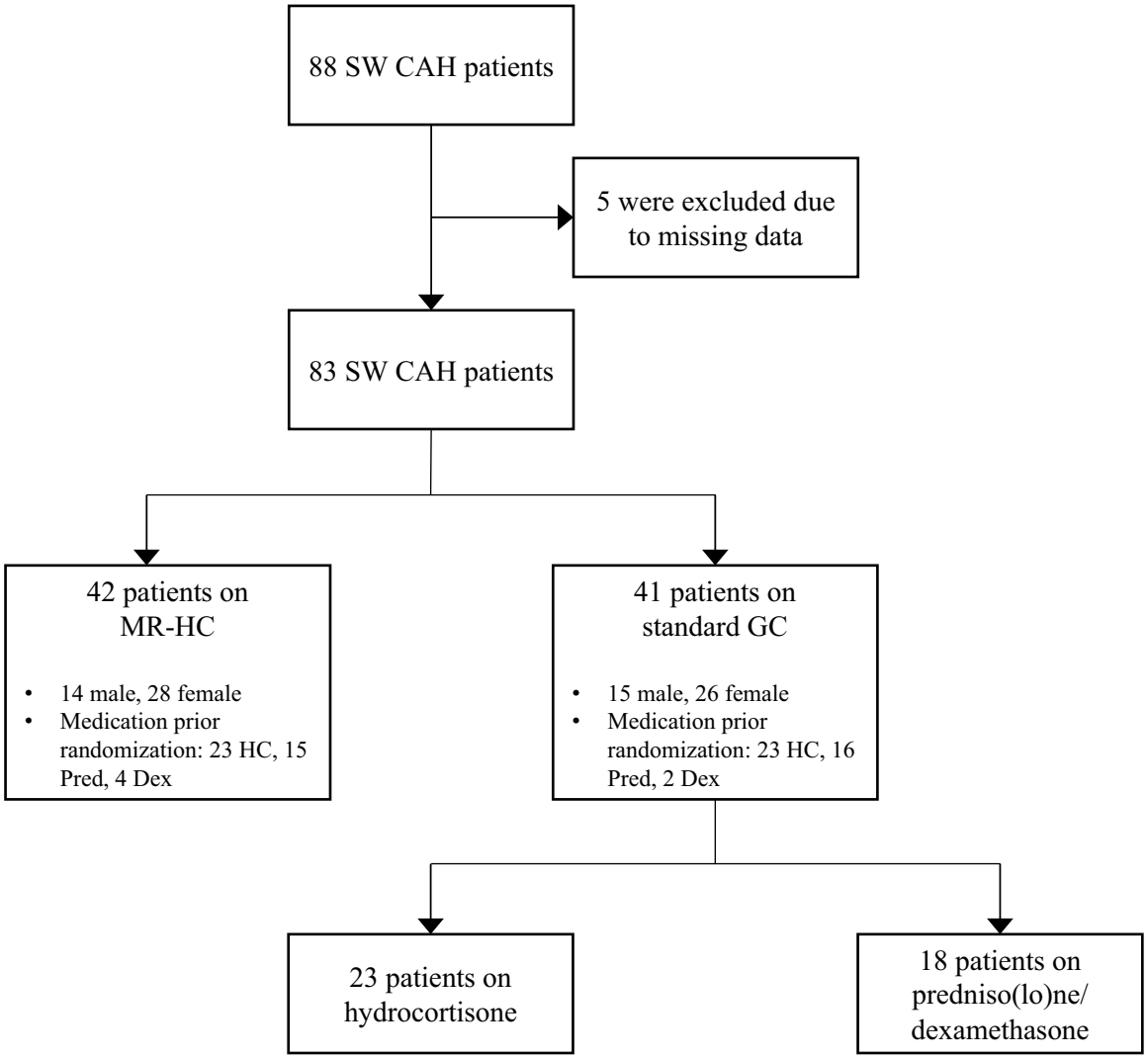

Figure 1. Flowchart of included patients with SW-CAH, randomized allocation in study arm and division into different subgroups with baseline characteristics of patients on modified-release hydrocortisone (MR-HC) and standard glucocorticoid (GC) therapy. Abbreviations: Dex, dexamethasone; HC, hydrocortisone; Pred, prednisolone.

Table 1. Baseline characteristics of treatment groups

		MR-HC	Standard GC	HC	Predniso(lo)ne/Dexamethasone
		$n = 42$	$n = 41$	Subgroup $n = 23$	Subgroup $n = 18$
Age in years	Median (range)	33.0 (31.0)	37.0 (47.0)	29.0 (38.0)	41.5 (43.0)
BMI		26.7 (25.8)	27.0 (17.2)	26.9 (17.2)	27.3 (14.6)
GC dose in HC dose equivalent (mg d^{-1})		25.0 (35.0)	25.0 (65.0)	22.5 (30.0)	30.0 (64.4)
MC dose (mg d^{-1})		0.100 (0.375)	0.100 (0.375)	0.075 (0.225)	0.100 (0.350)
	N (%)				
Female sex		28 (66.7)	26 (63.4)	14 (60.9)	12 (66.7)
Prior therapy					
HC		23 (54.8)	23 (56.1)	23 (100)	
Pred		15 (35.7)	16 (39.0)		16 (88.9)
Dex		4 (9.5)	2 (4.9)		2 (11.1)

Data are expressed as median (interquartile range) and number (percentage).
Abbreviations: BMI, body mass index; Dex, dexamethasone; GC, glucococrticoid; HC, hydrocortisone; MC, mineralocorticoid; MR-HC, modified-release hydrocortisone; Pred, prednisolone;.

Table 2. Correlation analysis of included patients with SW-CAH at the baseline.

	GC dose	dBP	sBP	Na	K	PRA	A4	17OHP	MC dose
GC dose									
dBP	0.04								
sBP	−0.03	0.73[a]							
Na	0.04	−0.05	0.06						
K	0.01	−0.08	−0.19	−0.19					
PRA	−0.05	0.02	−0.05	−0.44[a]	0.44[a]				
A4	−0.03	−0.11	0.08	−0.07	0.20	0.20			
17OHP	−0.08	−0.11	0.01	−0.11	0.24[b]	0.27[b]	0.87[b]		
MC dose	0.28[b]	0.12	0.13	0.00	−0.23[b]	−0.04	0.09	−0.06	

Abbreviations: 17OHP, serum 17alpha-hydroxyprogesterone; A4, serum androstenedione; dBP, diastolic blood pressure; GC, glucocorticoid; K, serum potassium; MC, mineralocorticoid; Na, serum sodium; PRA, plasma renin activity; sBP, systolic blood pressure.
[a]$P \leq .01$. [b]$P \leq .05$.

Table 3. Correlation analysis of included patients with SW-CAH at week 24.

	GC dose	dBP	sBP	Na	K	PRA	A4	17OHP
GC dose								
dBP	−0.12							
sBP	0.22[a]	0.53[b]						
Na	0.12	−0.01	0.22					
K	0.06	−0.01	−0.05	−0.24[a]				
PRA	0.01	−0.03	−0.09	−0.42[b]	0.57[b]			
A4	0.30[b]	−0.12	0.16	0.10	−0.06	0.09		
17OHP	0.16	−0.14	0.12	0.05	0.05	0.19	0.78[b]	

Abbreviations: 17OHP, serum 17alpha-hydroxyprogesterone; A4, serum androstenedione; dBP, diastolic blood pressure; GC, glucocorticoid; K, serum potassium; MC, mineralocorticoid; Na, serum sodium; PRA, plasma renin activity; sBP, systolic blood pressure.
[a]$P \leq 0.05$. [b]$P \leq .01$.

Table 4. Characteristics of patients with SW-CAH in the subgroups on modified-release hydrocortisone (MR-HC) versus standard glucocorticoid (GC) therapy at baseline and week 24.

		MR-HC	Standard GC	HC	Predniso(lo)ne/Dexamethasone	*P*
		n = 42	*n* = 41	Subgroup *n* = 23	Subgroup *n* = 18	
Median MC dose (mg d^{-1})	Baseline	0.100 (0.141)	0.100 (0.084)	**0.075 (0.050)**	**0.100 (0.100)**	**0.048**[a]
	24 weeks	0.100 (0.141)	0.100 (0.084)	0.075 (0.050)	0.100 (0.100)	
Median GC dose in HC dose equivalent (mg d^{-1})	Baseline	**25.0 (10.0)**	25.0 (11.3)	**22.5 (8.0)**	**30.0 (16.3)**	**0.042**[b] **0.005**[a]
	24 weeks	25.0 (20.0)	31.3 (15.0)	25.0 (20.0)	35.0 (11.7)	
Mean Na (mmol L^{-1})	Baseline	**138.8 (1.9)**	**139.8 (1.6)**	140.0 (1.2)	139.6 (2.0)	**0.020**[c]
	24 weeks	139.3 (1.8)	139.3 (1.9)	139.7 (2.0)	138.9 (2.0)	
Median 17OHP (nmol L^{-1})	Baseline	61.5 (161.1)	27.4 (139.1)	44.2 (181.1)	20.1 (82.6)	
	24 weeks	**2.5 (8.3)**	**10.5 (55.2)**	**11.7 (70.7)**	8.3 (55.1)	**0.001**[c] **0.006**[d]

Data are expressed as mean (standard deviation) for normally distributed data or as median (interquartile range) for non-normally distributed data. Significant differences are indicated in bold.
Abbreviations: 17OHP, serum 17alpha-hydroxyprogesterone; Dex, dexamethasone; HC, hydrocortisone; MC, mineralocorticoid (=9α-fluorocortisol); MR-HC, modified-release hydrocortisone; Na, serum sodium; Pred, prednisolone.
[a]HC versus Pred/Dex.
[b]MR-HC versus Pred/Dex.
[c]MR-HC versus standard GC.
[d]MR-HC versus HC.

22.5 mg d^{-1} (IQR 8.0); $P = .005$) and to patients receiving MR-HC (30.0 mg d^{-1} (IQR 16.3) versus 25.0 mg d^{-1} (IQR 10.0); $P = .042$).

At week 24, the median serum 17OHP concentration was significantly lower in patients receiving MR-HC than in patients on standard GC (2.5 nmol L^{-1} (IQR 8.3) versus 10.5 nmol L^{-1} (IQR 55.2); $P = .001$).

When further divided in subgroups, median serum 17OHP concentration at week 24 was significantly lower in patients receiving MR-HC than in patients receiving normal HC (2.5 nmol L^{-1} (IQR 8.3) versus 11.7 nmol L^{-1} (IQR 70.7); $P = .006$).

Otherwise, the groups showed no significant difference at baseline or week 24 with regard to the outcome variables. An overview of significant differences between groups at baseline and week 24 is given in Table 4.

Changes within groups between baseline and week 24

Table 5 provides an overview of the basic descriptions of the outcome variables for all groups at baseline and week 24, as well as the significant changes within groups from baseline

Table 5. Overview of basic descriptive of outcome variables for all groups at baseline and 24 weeks, as well as significant changes within groups from baseline to 24 weeks.

	MR-HC			Standard GC			HC			Predniso(lo)ne/ Dexamethason		
	$n = 42$			$n = 41$			Subgroup $n = 23$			Subgroup $n = 23$		
	Baseline	24 weeks	p	Baseline	24 weeks	p	Baseline	24 weeks	p	Baseline	24 weeks	P
Median GC dose in hydrocortisone dose equivalent (mg d^{-1})	25.0 (10.0)	25.0 (20.0)	0.062	25.0 (11.3)	31.3 (15.0)	0.001	22.5 (8.0)	25.0 (20.0)	0.005	30.0 (16.3)	35.0 (11.7)	.057
Median MC dose (mg d^{-1})	0.100 (0.141)	0.100 (0.141)	1.000	0.100 (0.084)	0.100 (0.084)	0.317	0.075 (0.050)	0.075 (0.050)	0.317	0.010 (0.100)	0.010 (0.100)	1.000
Mean dBP (mmHg)	74.3 (11.2)	73.5 (9.8)	0.571	74.3 (9.4)	72.8 (9.0)	0.308	74.3 (9.8)	73.0 (10.3)	0.539	72.5 (12.3)*	72.7 (11.0)	.550
Mean sBP (mmHg)	121.7 (12.3)	121.2 (10.9)	0.803	123.2 (13.6)	121.2 (10.8)	0.224	126.1 (12.0)	122.0 (12.5)	0.068	119.5 (15.0)	120.1 (8.3)	.812
Mean Na (mmol L^{-1})	138.8 (1.9)	139.3 (1.8)	0.047	139.8 (1.6)	139.3 (1.9)	0.135	140.0 (1.2)	139.7 (2.0)	0.435	139.6 (2.0)	138.9 (2.0)	.210
Mean K (mmol L^{-1})	4.0 (0.4)*	4.1 (0.3)	0.139	4.0 (0.3)	4.1 (0.3)	0.165	4.1 (0.3)	4.1 (0.3)	0.847	4.0 (0.3)	4.2 (0.3)	.056
Median PRA (ng L^{-1} s^{-1})	0.83 (1.0)	0.48 (0.61)	0.012	0.53 (0.66)	0.52 (0.78)	0.613	0.58 (0.55)	0.52 (0.75)	0.831	0.48 (0.90)	0.51 (0.90)	.663
Median A4 (nmol L^{-1})	3.53 (9.88)	1.31 (2.28)	<0.001	2.27 (10.1)	1.71 (3.18)	0.003	4.43 (15.95)	2.02 (3.21)	0.014	2.02 (4.14)	1.45 (1.89)	1.33
Median 17OHP (nmol L^{-1})	61.5 (161.1)	2.5 (8.3)	<0.001	27.4 (139.1)	10.5 (55.2)	0.003	44.2 (181.1)	11.7 (70.7)	0.006	20.1 (82.6)	8.3 (55.1)	.199

Data are expressed as mean (standard deviation) for normally distributed data or as median (interquartile range) for non-normally distributed data. Values marked with * are given as median (interquartile range) contrary to the row description due to lack of normal distribution.
Abbreviations: MR-HC, modified-release hydrocortisone; GC, glucococrticoid; MC, mineralocorticoid; dBP, diastolic blood pressure; sBP, systolic blood pressure; Na, serum sodium; K, serum potassium; PRA, plasma renin activity; A4, serum androstenedione; 17OHP, serum 17-alpha-hydroxyprogesterone.

to week 24. Comparing baseline to week 24 in patients staying on their standard GC medication, we observed a significant increase in median hydrocortisone dose equivalent (25.0mg d^{-1} (IQR 11.3) versus 31.3 mg d^{-1} (IQR 15.0) ; $P = .001$) and a significant decrease in median A4 (2.27 nmol L^{-1} (IQR 10.1) versus 1.71 nmol L^{-1} (IQR 3.18) ; $P = .003$) and median 17OHP (27.4 nmol L^{-1} (IQR 139.1) versus 10.5 nmol L^{-1} (IQR 55.2); $P = .003$).

Patients receiving MR-HC showed a significant increase in mean serum sodium from baseline to week 24 (138.8 mmol L^{-1} (SD 1.9) versus 139.3 mmol L^{-1} (SD 1.8); $P = .047$) as well as a significant decrease in median concentrations of PRA (0.83 ng L^{-1} s^{-1} (IQR 1.0) versus 0.48 ng L^{-1} s^{-1} (IQR 0.61); $P = .012$), serum A4 (3.53 nmol L^{-1} (IQR 9.88) versus 1.31 nmol L^{-1} (IQR 2.28); $P < .001$) and serum 17OHP (61.5 nmol L^{-1} (IQR 161.1) versus 2.5 nmol L^{-1} (IQR 8.3); $P < .001$).

The additional performance of a repeated-measures ANCOVA for both groups including all four time points with regard to serum sodium and adjusted for gender and baseline serum sodium, using Greenhous-Geisser adjustment, also revealed a significant difference in serum sodium over time ($F(2.65, 203.74) = 0.04$, $P < .001$, partial $\eta^2 = 0.11$)). However, there was no statistically significant interaction between serum sodium and treatment group ($F(2.65, 203.74) = 0.04$, $P = .985$, partial $\eta^2 = 0.00$). Additionally, serum sodium at baseline proved to be a significant covariate (($F(2.65, 203.74) = 0.04$, $P < .001$, partial η^2=0.11)) contrary to gender (($F(2.65, 203.74) = 0.04$, $P = .221$, partial $\eta^2 = 0.02$)).

In linear regression models, the mean delta 17OHP (−82.6 (SD 141.5)) in the whole study population from baseline to week 24 was not a significant predictor for either serum sodium (β=−.16; $t = -1.43$; $P = .156$) nor PRA ($\beta = .09$; $t = 0.80$; $P = .429$) at week 24.

Patients in the standard GC group who stayed on HC showed a significant increase in median hydrocortisone dose equivalent from baseline to week 24 (22.5 mg d^{-1} (IQR 8.0) versus 25.0 mg d^{-1} (IQR 20.0); $P = .005$), as well as a significant decrease in median serum A4 (4.43 nmol L^{-1} (IQR 15.95) versus 2.02 nmol L^{-1} (IQR 3.21); $P = .014$) and 17OHP (44.2 nmol L^{-1} (IQR 181.1) versus 11.7 nmol L^{-1} (IQR 70.7); $P = .006$).

Patients who stayed on standard GC with predniso(lo)ne/ dexamethasone alone or in combination with another GC showed no significant changes from baseline to week 24 at all (Table 5).

Analysis excluding patients with antihypertensive medication and drospirenone-containing contraceptive

After excluding two patients who received antihypertensive medication and two on drospirenone-containing contraceptives, the results of this sub-analysis remained the same as in the analysis of the total sample, except for the no longer significant increase in mean serum sodium concentrations from baseline to week 24 in the MR-HC group.

Analysis of female patients

The sub-analysis of the 54 female patients in our cohort revealed roughly the same results as in the main analysis of the total sample, except for the following differences: At baseline, there was no significant difference in serum sodium levels between female patients who stayed on their standard GC

medication and those who received MR-HC. However, female patients who stayed on their standard GC medication were significantly older than those who received MR-HC. Comparing baseline to week 24 in female patients staying on their standard GC medication, we no longer observed a significant decrease of 17-OHP and A4 levels. In female patients receiving MR-HC we no longer observed a significant increase in serum sodium from baseline to 24 weeks.

Discussion

In a carefully controlled study with standardized collection of PRA we have confirmed that PRA correlated negatively with serum sodium and positively with serum potassium, reflecting the regulation in the RAA system,[7] and demonstrated that PRA positively correlates with serum 17OHP concentrations in our SW-CAH patients. We have shown that the improved biochemical control of 17OHP in the SW-CAH patients on MR-HC was associated with a decrease in PRA despite unchanged 9α-fluorocortisol dose. These results suggest that normalizing 17OHP might reverse the antagonistic action 17OHP has on the hMR and therefore increases the agonist action of 9α-fluorocortisol.

It has been suggested previously, but not demonstrated, that progesterone and its metabolites exacerbate MC deficiency in SW-CAH patients through antagonism at the hMR.[11,12] This correlates with reports that Addisonian women require higher 9α-fluorocortisol doses during pregnancy to maintain normal potassium levels.[17] In vitro studies investigated the agonistic and antagonistic properties of progesterone and its metabolites in CV-1 cells co-transfected with a hMR expression vector together with a luciferase reporter gene.[13] These studies revealed that 17OHP binds well to the hMR (Ki = 16.5 nmol L^{-1}), possesses only a weak agonistic effect (ED_{50} > 1000 nmol L^{-1}), but a strong antagonistic effect with an IC_{50} of 135 nmol L^{-1}.[13] Even at doses of 10 nmol L^{-1} 17OHP displaced up to 20% of aldosterone from the hMR.[13] These findings were verified with different concentrations of 17OHP using the hMR and the frequent hMR p.Ile180Val single nucleotide polymorphism[18] demonstrating that 20 nmol L^{-1} 17OHP inhibited 20%-25% and 250 nmol L^{-1} 17OHP more than 80% of aldosterone-induced hMR transactivation. Interestingly, translocation of the hMR to the cell nucleus was not inhibited by 17OHP,[18] which is described for other hMR antagonists such as spironolactone and eplerenone.[19] Therefore, it is assumed that the antagonistic effect of 17OHP binding to the hMR might be caused by a conversion to a transcriptionally inactive hMR conformation.[18] The hMR antagonistic effect of progesterone and its metabolite 17OHP has also been demonstrated in patients with primary adrenal insufficiency.[14]

The previously recommended target range of serum 17OHP in CAH patients is 12–36 nmol L^{-1},[20] however in a large national cohort study 43% of female classic and 52% of male CAH patients had serum 17OHP levels higher than 36 nmol L^{-1}, with more than 20% having levels higher than 100 nmol L^{-1}.[21] Together with the finding of the anti-hMR properties of 17OHP those findings explain the need of increased 9α-fluorocortisol doses in poorly controlled SW-CAH with high androgen precursors. In poorly controlled CAH patients not only highly increased serum 17OHP concentrations are found, but also other increased steroid precursors, such as progesterone and the partial hMR agonists 21-deoxycorticosterone (21-DB; 11-hydroxyprogesterone) and 21-deoxycortisol (21-DF).[22] Although we did not measure these compounds in our study, we assume that since the combination of 21-DF or 21-DB at 10^{-6} M with 10^{-9} M aldosterone significantly reduced hMR-mediated transactivation by 45% and 47%, respectively,[22] a general better control of steroid precursors in CAH patients using the MC-HC therapy, would also lower progesterone, 21-DF or 21-DB concentrations and their effect on the hMR. In the phase 3 MR-HC study patients were very carefully titrated by blinded titrators such that in the MR-HC group the serum 17OHP levels were effectively normalized (<10 nmol L^{-1}) throughout the 24 hours in the majority of patients and lower than those in the standard treatment group. This was associated with a fall in PRA in the MR-HC group compatible with the in vitro data showing that >10 nmol L^{-1} 17OHP can have an antagonistic action at the hMR.[13]

In the initial analysis, we were also able to show a significant increase in serum sodium from baseline to week 24 in the MR-HC group, but not the standard GC group. In further analyses, however, this effect was not associated with treatment group. Therefore, further studies with a larger number of patients are needed to investigate serum sodium under MR-HC.

We demonstrated that the MR-HC group showed a significant decrease in PRA from baseline to week 24, without a significant change in GC or MC dose from baseline to week 24. Although these observed changes remained within the laboratory normal range, this observed increase in MC activity might explain the unexpected occurrence of carpal tunnel syndrome in three patients in the phase 2 study and five patients in the phase 3 study of MR-HC, leading to discontinuation of MR-HC in one of the patients in the phase 3 study.[2,3]

Since antihypertensive drugs, contraceptive medications and the menstrual cycle interfere with the RAA system, especially with PRA, we performed a sub-analysis by excluding patients on those medications and a sub-analysis of our female cohort, confirming these findings.

The predniso(lo)ne/dexamethasone group did not show a significant change in PRA from baseline to week 24 and tended to have lower PRA levels at baseline than the MC-HC group. This is probably due to the significantly higher median 9α-fluorocortisol dose at baseline in those patients receiving predniso(lo)ne/dexamethasone. Those patients also received a significantly higher median hydrocortisone dose equivalent compared to patients on HC at baseline which consequently resulted in lower serum 17OHP concentrations in patients on predniso(lo)ne/dexamethasone therapy than with conventional HC therapy. Interestingly, the PRA concentrations did not significantly differ between the two groups which implies that the higher 9α-fluorocortisol dose in the predniso(lo)ne/dexamethasone group is probably also due to the lower intrinsic MC potency of synthetic GCs.[23]

A more efficacious and lower-dose 9α-fluorocortisol replacement under MR-HC treatment might result in clinical benefit in the long term. In regard to these results, it may be necessary to change the current monitoring target from still elevated 17OHP levels (<36 nmol L^{-1}) to normalization of 17OHP levels in patients with SW-CAH, leading to lower 9α-fluorocortisol doses in the future. However, it should be stressed that lower 17-OHP levels should not be achieved by higher GC doses used, but by better circadian application of GCs thus imitating the physiological cortisol secretion. In

the long term, this may reduce cardiovascular risk in SW-CAH patients.[24] Also, stabilizing water and electrolyte homeostasis in situations with low blood pressure and gastrointestinal electrolyte loss may decrease the incidence of adrenal crisis[25,26] and hospitalizations. Finally, the mood has been shown to be better during high MR occupation (after 9α-fluorocortisol intake) compared to low MR occupation (without 9α-fluorocortisol intake) in patients with adrenal insufficiency.[27]

Limitations of the study include that we did not account for menstrual cycle status in women with SW-CAH, therefore we cannot rule out an effect of the luteal phase on PRA. However, a sub-analysis of our female cohort confirmed our findings. Furthermore, we investigated only morning blood samples. It would be interesting to investigate diurnal variation in 17OHP levels and their effect on PRA throughout the day. The strength of the study was that the MC replacement therapy using 9α-fluorocortisol dose remained stable across the study period, which allowed us to study further influences on PRA besides the effect of the mineralocorticoid 9α-fluorocortisol itself. Secondly, the rigid study protocol with sample collection in a supine position in the morning, similar procedures undertaken at all study visits and all study sites, a centralized hormone analysis, no significant change in body weight and diet during the study ruled out significant effects of these potentially confounding factors on PRA measurement. However, any medication (eg, NSAIDs, food and mineral supplements, SSRI) considered necessary for the subject's safety and well-being could be given during the study at the discretion of the investigator(s). Therefore, we cannot rule out effects of these medications on the RAAS. In summary, we have shown for the first time that 6 months of MR-HC therapy is associated with a decrease in PRA and an increase in serum sodium, indicating a greater agonist action of the 9α-fluorocortisol dose, likely due to the efficient lowering of the circulating concentrations of the MC-receptor antagonist 17OHP.

Declaration of interest

The authors have the following conflicts of interest to declare in relation to this work: N.R., W.A., A.B.P., A.L.H., A.J., A.M., J.N.P., C.P., A.P., D.A.R., N.M.M.L.S., and P.To. were study investigators. D.P.M has received research funds from Diurnal Ltd through an NIH Cooperative Research and Development Agreement. R.J.R. is a director, and J.P. and H.C. are employees, of Diurnal Ltd. L.T., M.Q. reports no conflict of interest. A.M. is currently employed at AstraZeneca, Gaithersburg, USA. Co-authors W.A. and N.R. are on the editorial board of EJE. They were not involved in the review or editorial process for this paper, on which they are listed as authors.

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