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ORIGINAL ARTICLE

CYP11B2 inhibitor dexfadrostat phosphate suppresses the aldosterone-to-renin ratio, an indicator of sodium retention, in healthy volunteers

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Aims: High aldosterone is a key driver of hypertension and long-term negative sequelae. We evaluated the safety and efficacy of dexfadrostat phosphate (DP13), a novel aldosterone synthase (CYP11B2) inhibitor, in healthy participants.

Methods: This randomized, double-blind, placebo-controlled study was conducted in two parts. In part A, a single-ascending dose escalation, 16 participants received oral DP13 1–16 mg. Part B was a multiple-ascending dose, sequential group study in which 32 participants received oral DP13 4, 8 or 16 mg once daily for 8 days. Safety and tolerability were monitored throughout. An adrenocorticotropic hormone (ACTH) stimulation test at maximal blood drug concentrations defined the dose range for multiple dosing.

Results: DP13 was well tolerated at all doses, with no serious adverse events. In part B, all DP13 doses (4, 8 and 16 mg) over 8 days effectively suppressed aldosterone production, increased the urinary sodium/potassium ratio, decreased plasma sodium and increased plasma potassium and renin levels compared with placebo, resulting in potent suppression of the aldosterone-to-renin ratio (ARR). Endocrine counter-regulation resulted in the 4 mg dose no longer sustaining 24-h aldosterone suppression after 8 days of treatment, unlike the 8- and 16 mg doses. There was no evidence of drug-induced adrenal insufficiency (ACTH stress challenge).

Conclusions: In patients with excess aldosterone and ensuing sodium retention driving hypertension, managing sodium balance is critical. A CYP11B2 inhibitor like DP13, whose effectiveness can be monitored by a reduction in ARR, may prove valuable in managing aldosterone-dependent hypertension and primary aldosteronism.

KEYWORDS
aldosterone-to-renin ratio, aldosterone, aldosterone synthase inhibition, CYP11B2, healthy volunteers, renin, sodium/potassium balance

The authors confirm that the Principal Investigator for this paper is Prof. Paolo Mulatero and that he had direct clinical responsibility for patients.

1 | INTRODUCTION

The renin–angiotensin–aldosterone system (RAAS) is an essential hormonal system controlling the homeostasis of electrolytes, fluids and blood pressure via a biofeedback loop.[1] Volume depletion and hypoperfusion of the kidney activate the RAAS to mediate vasoconstriction and conserve sodium to actively retain water, resulting in volume expansion. Aldosterone, produced by the adrenal gland, regulates fluid homeostasis by stimulating sodium reabsorption in exchange for potassium in the distal tubules and collecting duct of the kidney.[2,3] Inappropriately elevated aldosterone levels in the setting of high sodium status have been associated with hypertension and tissue fibrosis leading to remodelling and disease including arterial hypertension, cardiac arrhythmias, heart failure and stroke.[4]

Importantly, aldosterone has been demonstrated to affect morbidity and mortality in patients with primary aldosteronism (PA) at least partially independent of blood pressure.[5–7] A once-daily oral treatment that would produce biochemical correction resulting in clinical correction of PA through direct effect on the source of aldosterone synthesis might offer a treatment option for a broad range of patients with PA.

Aldosterone synthase, encoded by the CYP11B2 gene,[8,9] is the rate-limiting enzyme for aldosterone biosynthesis, catalysing three consecutive reactions to convert deoxycorticosterone (DOC) to aldosterone. A new pharmacological approach to suppress aldosterone came with the finding that the racemic, non-steroidal aromatase (CYP19A1) inhibitor CGS16949A (FAD286) demonstrated CYP11B2 inhibition.[10,11] Subsequent chromatographic purification of the individual enantiomers revealed the laevorotatory CGS20287 enantiomer inhibited CYP19A1 200-fold more potently than the dextrorotatory CGS20286 enantiomer.[12] Recognizing that CGS20286 inhibited aldosterone in vitro, it was evaluated in rodent disease models and shown to reduce aldosterone, inflammation, fibrosis and end-organ damage in the heart and kidneys.[13,14] While FAD286 established the preclinical concept of CYP11B2 inhibition, the compound's pharmacological selectivity, dosing regimen, preparative purity and physicochemical stability presented an obstacle for clinical development. Therefore, a new technical process was established to derive from the original racemate, via enantioselective crystallization, the novel investigational drug dexfadrostat phosphate (DP13) (US Patent No. 10822332 B2).[15] DP13 is non-hygroscopic over a wide humidity range and stable over time with respect to enantiomeric purity and polymorphic form, making it a compelling agent for clinical development. The chiral purity of DP13 revealed an enantiomeric excess of 99.9% eliminating nearly all the CYP19A1 activity.

Inhibiting CYP450 enzymes as a means of selectively inhibiting even terminal enzymes involved in steroidogenesis has proven difficult. With respect to CYP11B2, it is particularly important to avoid excessive inhibition of CYP11B1 (responsible for cortisol synthesis) and/or CYP19A1 (responsible for oestrogen synthesis). Inhibition of CYP11B1 will suppress the adrenal stress response while inhibition of CYP19A1 will disrupt the regulation of the hypothalamic–pituitary–gonadal (HPG) axis. Furthermore, excessive inhibition of the conversion of DOC to corticosterone by inhibiting either CYP11B1 or CYP11B2 can result in the excessive accumulation of DOC, a mineralocorticoid that can lessen the benefits of inhibiting aldosterone production. A favourable pharmacodynamic (PD) profile would avoid inhibiting CYP11B1, CYP19A1 or CYP11B2 in a manner that compromises the adrenal stress response or gonadal sex hormone production or leads to excessive DOC production at doses that achieve maximal therapeutic inhibition of aldosterone synthesis; the pharmacokinetic (PK) profile should provide a dose-dependent balanced pharmacology over 24 h with chronic once-daily dosing.[16]

Here, we report a phase 1 trial investigating DP13 as a CYP11B2 inhibitor in healthy male volunteers. The trial consisted of a single-ascending dose study (part A) designed to select the dosing regimen for a multiple-ascending dose study (part B) to characterize the dose-dependent safety, efficacy and selectivity profile of DP13.

What is already known about this subject

- Early clinical profiling of aldosterone synthase (CYP11B2) inhibitor compounds revealed unpredictable pharmacology for pharmacokinetic-dependent efficacy and selectivity and have not been successful to date.
- Inhibitors of steroidogenesis often have cross-reactivity with other CYP450 enzymes that regulate hormone synthesis and drug metabolism.

What this study adds

- Dexfadrostat phosphate (DP13) demonstrates dose-dependent reduction of the aldosterone-to-renin ratio, a marker of sodium retention, without affecting the adrenal stress response or the hypothalamic–pituitary–gonadal axis.
- DP13 inhibits all three enzymatic conversions mediated by aldosterone synthase leading to moderate, physiologically insignificant substrate accumulation.
- DP13 resets the sodium/potassium balance to increase plasma potassium without causing hyperkalaemia.

2 | METHODS

2.1 | Objectives

This first-in-human study aimed to determine the therapeutic window for aldosterone suppression and assess the safety and tolerability of DP13 in healthy male participants. The primary objectives were to

determine the safety and tolerability of single and multiple oral doses of DP13 and the PD of aldosterone suppression. The secondary objectives were to determine the PK of single and multiple oral doses of DP13, as well as any dose-dependent effect on cortisol excursion or sex hormone synthesis.

2.2 | Study design

This single-centre, randomized, double-blind, placebo-controlled study was conducted in two parts between 20 March 2017 and 9 February 2018. Part A was a single-ascending dose study using an alternating group, three-period design, to inform the dose regimen used in part B, which was a multiple-dose, sequential group study.

The study was carried out in accordance with the Declaration of Helsinki, the Good Clinical Practice Guideline of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, the European Medicines Agency Guidelines for Good Clinical Practice and Good Manufacturing Practice. The study was registered prior to patient enrolment (ClinicalTrials.gov unique identifier: NCT03046589; EudraCT 2016-003648-36). Approval for this study was granted by the London—Brent Research Ethics Committee (NHS Health Research Authority; Reference #16/LO/1825) and the Medicines and Healthcare products Regulatory Agency (Reference #47238/0001/001-0001). All participants freely gave their written informed consent before starting the study.

2.3 | Participants

Men aged 18–45 years with a body mass index of 18–30 kg m^{-2} and a body weight of 60–95 kg were enrolled. Participants met the inclusion criteria if they were in good health, had normal **cortisol** excursions (>20 μg dL^{-1} or >552 nmol L^{-1}) after **adrenocorticotropic hormone** (ACTH) stress test and had serum sodium and potassium levels within the normal laboratory reference ranges. Participants were excluded if they had previously received any medication in the 30 days before the first dose of the study drug that was known to chronically alter drug absorption or elimination or had received any systemic or topical medication potentially interfering with the study procedures in the 14 days before the first dose of the study drug. Other exclusion criteria included abnormalities in heart rate, electrocardiogram (ECG) or blood pressure, based on investigator clinical judgement, and renal impairment defined as a serum creatinine concentration above 177 μmol L^{-1}.

2.4 | Interventions

In study part A (Figure 1A), participants were randomized to one of two placebo-controlled treatment groups to receive alternating escalating doses of DP13. Within each treatment group, participants were further randomized to receive DP13 or placebo at each escalation. Group A received DP13 1, 4 and 12 mg or placebo. Group B received 2, 8 and 16 mg DP13 or placebo. This dose range was selected based on prior clinical studies of racemic CGS16949A containing 50% of the DP13 free base, in which exposure to CGS16949A 1 or 4 mg was well tolerated in patients with breast cancer[17,18]; CGS16949A 4 mg suppressed aldosterone production without effect on serum cortisol in healthy men[19] and twice daily dosing of CGS16949A 8 mg produced modest but clinically insignificant blunting of cortisol response in women with breast cancer.[20] A minimum of 6 days between each dose escalation per group and a minimum of 13 days between doses for each participant were followed to allow review of the blinded safety, PK and PD data.

Study part B was a multiple-dose, sequential group study (Figure 1B), with the DP13 dose range informed by the outcome of part A. Participants were randomized to three placebo-controlled dose groups receiving DP13 4, 8 or 16 mg once daily (QD) for 8 days. There was a minimum of 6 days between each dose escalation step.

In both study parts, all doses were administered in the morning as oral capsules to participants in the fasted state. Throughout each study part, participants followed a World Health Organization-recommended sodium/potassium-standardized diet (sodium intake: 70–80 mEq [1.6–1.8 g] day^{-1}; potassium intake 80–90 mEq [3.1–3.5 g] day^{-1}).

2.5 | Safety and tolerability assessments

Safety and tolerability were monitored from the time of signing an informed consent form to final discharge from the study. Assessments included the monitoring of adverse events (AEs), vital signs, 12-lead ECGs, telemetry, 12-lead Holter, clinical laboratory evaluations and physical examination. Details of the classification of AEs can be found in the supporting information (S1).

2.6 | PD measurements

PD measurements assessed the plasma or urine concentrations of aldosterone, cortisol, DOC, **corticosterone**, renin, ACTH, **oestradiol**, **testosterone**, **follicle-stimulating hormone** (FSH), **luteinizing hormone** (LH) and electrolytes.

During study part A, blood samples for PD analysis were taken prior to each dose and at 6, 12, 24 and 48 h post-dose for aldosterone; 12, 24 and 48 h post-dose for cortisol; 1.5, 12 and 24 h post-dose for DOC and corticosterone; 6 h post-dose for sex hormones; 6 and 48 h post-dose for electrolytes; and 1.5 and 24 h post-dose for ACTH.

For study part B, blood samples were taken for all analytes prior to dosing on Days 1 and 8. Pre-dose samples were also taken on Day 2 for all analytes except renin. There were no additional post-dose time points for sex hormone analysis. For aldosterone, post-dose samples were taken at 6 and 12 h on Day 1 and at 6, 12 and 24 h on Day 8. For cortisol, post-dose samples were

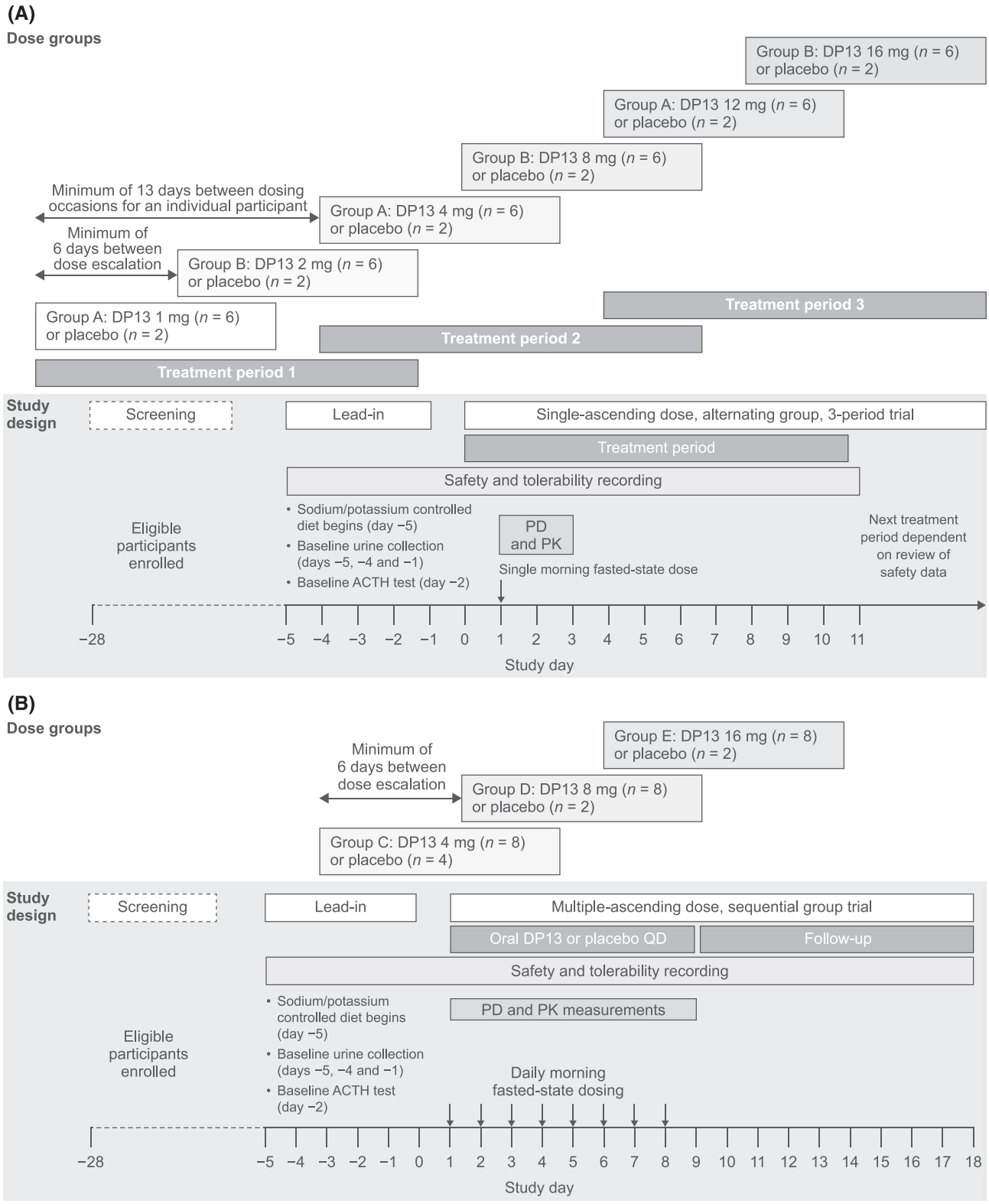


FIGURE 1 Study design for study part A (A) and study part B (B). ACTH, adrenocorticotropic hormone; DP13, dexfadrostat phosphate; PD, pharmacodynamics; PK, pharmacokinetics; QD, once daily.

taken at 12 h on Day 1 and at 12 and 24 h on Day 8. For DOC and corticosterone, post-dose samples were taken at 12 h on Day 1 and at 12 and 24 h on Day 8. For electrolytes and renin activity, post-dose samples were taken at 6 h on Days 1 and 8. For ACTH, a post-dose sample was taken at 24 h after the Day 8 dose. Urine sampling was conducted over 24 h on Days −5, −4 and −1 and for 24 h post-dose on Day 1 (part A) and Days 1, 7 and 8 (part B).

For the ACTH stress test, blood samples were taken 30 min prior to and 1 h after a single intravenous injection of **tetracosactide acetate** 250 μg (Synacthen, Mallinckrodt Pharma) to the arm. Blood was analysed for aldosterone and cortisol. The ACTH stress test was performed on Day −2 for baseline measurements and 2 h post-dose on Day 1 (part A) or Day 7 (part B).

PD markers in plasma and urine were measured using validated techniques in accordance with the manufacturers' instructions (see supporting information S2).

2.7 | PK measurements

PK parameters assessed in both parts of the study included the following: area under the concentration–time curve from time 0 to time of last quantifiable concentration ($AUC_{0\text{-}t}$), area under the concentration–time curve from time 0 extrapolated to infinity ($AUC_{0\text{-}\infty}$), maximal plasma concentration (C_{max}), time to reach maximal plasma concentration (T_{max}), plasma terminal elimination half-life ($t_{1/2}$), total plasma clearance (CL/F) and volume of distribution during terminal phase (Vz/F). Additional assessments included in part B were area under the concentration–time curve from time 0 to 24 h (AUC_{0-24}), minimum plasma concentration (C_{min}), accumulation ratio and linearity ratio.

During study part A, blood samples were taken for PK analysis prior to dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36 and 48 h post-dose in each treatment period. For study part B, blood samples were taken prior to each dose on Days 1 to 8 and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 16 h post-dose on Days 1 and 8 only. An additional sample was taken at 24 h post-dose on Day 8.

2.8 | Data and statistical analysis

Summary statistics were described for safety, tolerability, PD and PK data.

The PK population included all participants who received at least one dose of DP13 and had evaluable PK data. PK parameters were determined from plasma concentrations of DP13 using noncompartmental methods. $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$ and C_{max} were assessed for dose proportionality on Day 1 in part A and $AUC_{0\text{-}t}$ and C_{max} on Day 8 in part B. Least squares (LS) means for each dose level were calculated. *P*-values for overall and pairwise treatment comparisons are presented. Statistical analysis was used to determine pooled estimates (across the dose levels analysed) of the between-participant variability in the PK parameters.

The PD population included all participants who received at least one dose of DP13 or placebo and had evaluable PD data. Mixed models were used to analyse changes in stimulated steroid hormones from pre- to post-ACTH injection and changes in steroid levels from baseline and to perform the analyses of area under the concentration–time curve (AUC) from time 0 to 48 h post-dose (AUC_{0-48}) of aldosterone in part A and AUC_{0-24} of aldosterone, potassium and sodium on Days 1 and 8 in part B. LS means for each treatment at each time point were calculated along with the difference in LS means for each dose level *vs.* placebo and Day 8 *vs.* Day 1 and the associated 95% confidence intervals (CIs) and *P*-values. These were back-transformed to provide geometric LS means, a point estimate and 95% CI for the ratio of the geometric means. Changes in hormone concentration from pre- to post-ACTH injection, changes from baseline and Day 8 treatment comparisons were analysed using paired *t*-tests.

The safety populations consisted of all participants who received at least one dose of DP13 or placebo and had at least one post-dose safety assessment. All AEs were listed. Treatment-emergent AEs (TEAEs) were summarized by treatment, severity, relationship to the study drug and relationship to the ACTH stress test.

All statistical testing was conducted at an alpha level of 0.05. No adjustments for multiple comparisons were made. Calculations were performed using Phoenix WinNonlin (Certara USA Inc., Version 6.4). Additional information on the statistical analysis can be found in the supporting information (S3).

2.9 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[21]

3 | RESULTS

3.1 | Baseline demographics

In total, 48 healthy male participants were enrolled in and completed the study: 16 in part A and 32 in part B (Figure S1). The mean ages of participants in part A and part B were 36 and 30 years, respectively. Most participants were white (81.3% in part A and 90.6% in part B) (Table S1).

3.2 | Study part A

During part A, there were no serious AEs, and no participants discontinued the study. Of the 16 participants, 12 (75%) reported 26 TEAEs; most TEAEs were mild and three were moderate (Table 1). Four TEAEs were considered treatment related. Nausea ($n = 8$), headache ($n = 4$) and dizziness ($n = 3$) were the most frequently reported TEAEs. There was no association between the number of TEAEs and DP13 dose or placebo.

At all doses of DP13, the mean aldosterone AUC_{0-48} was significantly lower than placebo ($P < 0.001$ for all doses, Figure 2A). Compared with participants who received placebo, ACTH-stimulated aldosterone production was inhibited in patients who received DP13 at any dose ($P < 0.0001$ for all doses, Figure 2B). The duration of aldosterone suppression was dose-dependent, with serum aldosterone in participants receiving DP13 1 mg or 2 mg returning to

TABLE 1 Treatment-emergent adverse events during study parts A and B.

	Part A								Part B				
	Placebo $n = 12$	1 mg DP13 $n = 6$	2 mg DP13 $n = 6$	4 mg DP13 $n = 6$	8 mg DP13 $n = 6$	12 mg DP13 $n = 6$	16 mg DP13 $n = 6$	Overall $n = 16$	Placebo $n = 8$	4 mg DP13 QD $n = 8$	8 mg DP13 QD $n = 8$	16 mg DP13 QD $n = 8$	Overall $n = 32$
Number of TEAEs, *n*	5	3	6	2	5	2	3	26	7	9	11	12	39
Participants with TEAEs, *n* (%)	5 (41.7)	1 (16.7)	4 (66.7)	2 (33.3)	3 (50.0)	2 (33.3)	3 (50.0)	12 (75.0)	5 (62.5)	7 (87.5)	6 (75.0)	4 (50.0)	22 (68.8)
Severity, *n* (%)[a]													
Mild	4 (33.3)	1 (16.7)	3 (50.0)	2 (33.3)	3 (50.0)	2 (33.3)	2 (33.3)	11 (68.8)	5 (67.5)	7 (87.5)	4 (50.0)	4 (50.0)	20 (62.5)
Moderate	1 (8.3)	0	1 (16.7)	0	0	0	1 (16.7)	2 (12.5)	0	0	3 (37.5)	0	3 (9.4)
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0
Frequent TEAEs[b], *n* (%)[a]													
Abdominal discomfort	-	-	-	-	-	-	-	-	1 (12.5)	1 (12.5)	1 (12.5)	2 (25.0)	5 (15.6)
Cough	-	-	-	-	-	-	-	-	-	2 (25.0)	1 (12.5)	-	3 (9.4)
Dizziness	-	-	1 (16.7)	1 (16.7)	-	-	-	2 (12.5)	-	2 (25.0)	-	1 (12.5)	3 (9.4)
Flushing	-	-	-	-	-	-	-	-	1 (12.5)	-	-	1 (12.5)	2 (6.3)
Headache	1 (8.3)	-	1 (16.7)	-	1 (16.7)	-	1 (16.7)	3 (18.8)	1 (12.5)	1 (12.5)	1 (12.5)	2 (25.0)	5 (15.6)
Nasopharyngitis	-	-	-	-	-	-	-	-	1 (12.5)	-	3 (37.5)	-	4 (12.5)
Nausea	3 (25.0)	-	2 (33.3)	1 (16.7)	-	1 (16.7)	1 (16.7)	6 (37.5)	-	-	-	-	-
Oropharyngeal pain	-	-	-	-	-	-	-	-	1 (12.5)	-	1 (12.5)	-	2 (6.3)

Abbreviations: DP13, dexfadrostat phosphate; QD, once daily; TEAE, treatment-emergent adverse event.

[a]Number of participants.

[b]Frequent TEAEs are defined as those experienced by >2 participants in any group.

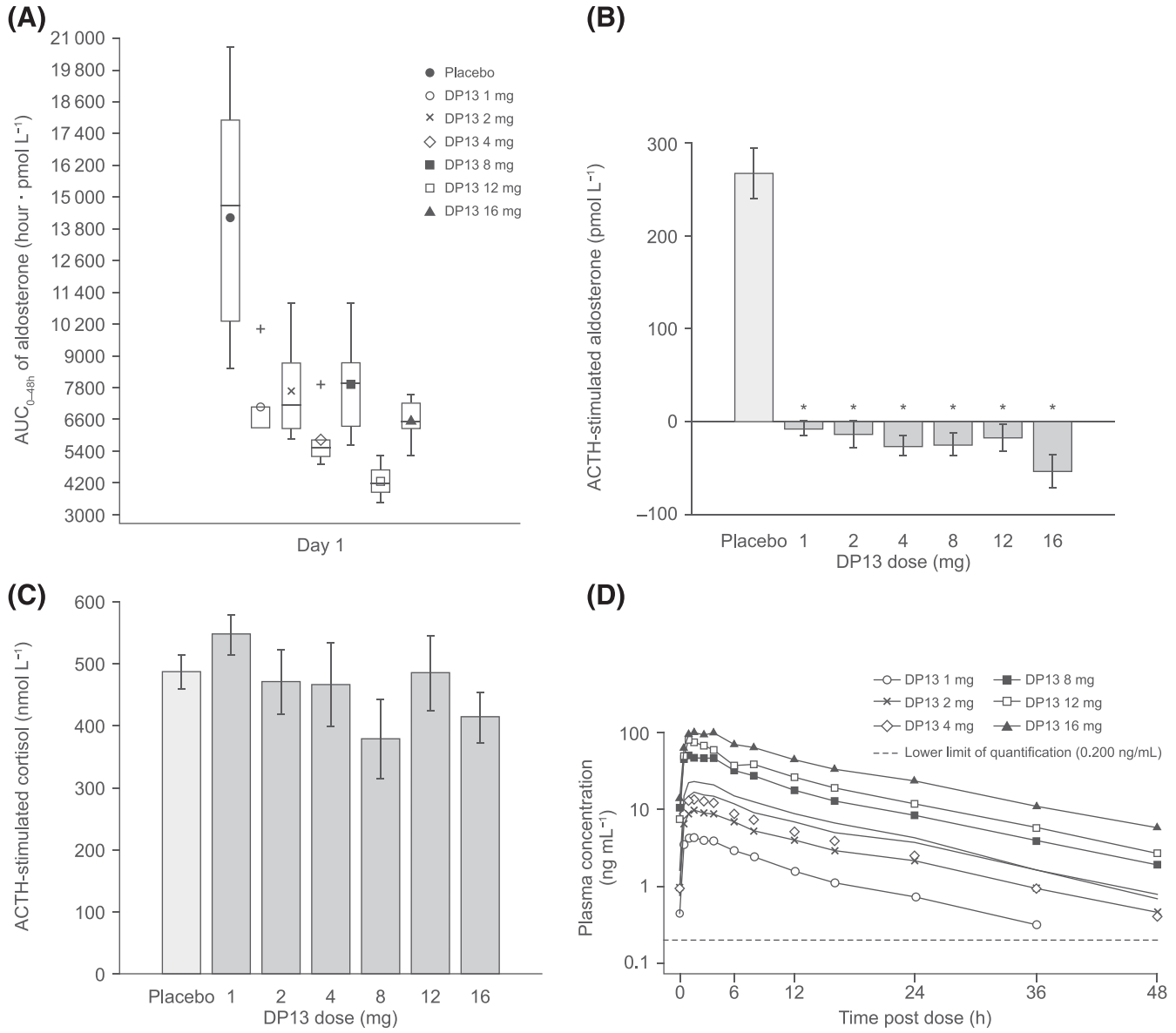


FIGURE 2 PD and PK data during study part A. AUC_{0-48} plasma aldosterone following single doses of DP13 or placebo (A), baseline-subtracted excursions of aldosterone (B) and cortisol (C) on ACTH stimulation test, and plasma concentration of DP13 following a single dose (D). Box plots in (A) represent the range (whiskers), 75% and 25% quartiles (box), median (midline), mean (key symbols), and outliers (+). *$P < 0.0001$ *vs.* placebo. ACTH, adrenocorticotropic hormone; AUC_{0-48}, area under the concentration–time curve from time 0 to 48 h post-dose; DP13, dexfadrostat phosphate; PD, pharmacodynamics; PK, pharmacokinetics.

baseline levels within 48 h. In contrast, over the entire DP13 dose range, baseline-subtracted cortisol stimulation was in the region of 378–547 nM and showed no dose-dependent blunting compared with placebo (Figure 2C).

Following a single oral dose, DP13 reached maximal plasma concentration (geometric mean C_{max} range across doses, 4.61–61.9 ng mL^{-1}) after approximately 2 h (T_{max} range across doses, 1.25–4.00 h) (Figure 2D). After reaching C_{max}, plasma concentrations declined in a monophasic manner with $t_{1/2}$ ranging from 9.5 to 11.1 h across doses. The dose-normalized DP13 $AUC_{0-\infty}$ and C_{max} were comparable over the entire dose range (normalized geometric mean $AUC_{0-\infty}$ range, 43.6–65.9; normalized C_{max} range, 3.47–5.73). The Vz/F and the CL/F were dose-independent (Vz/F range, 235–345 L; CL/F range, 253–382 mL min^{-1}).

Based on these safety, PD and PK assessments, doses of DP13 4, 8 and 16 mg QD were selected for the multiple-ascending dose study (part B).

3.3 | Study part B

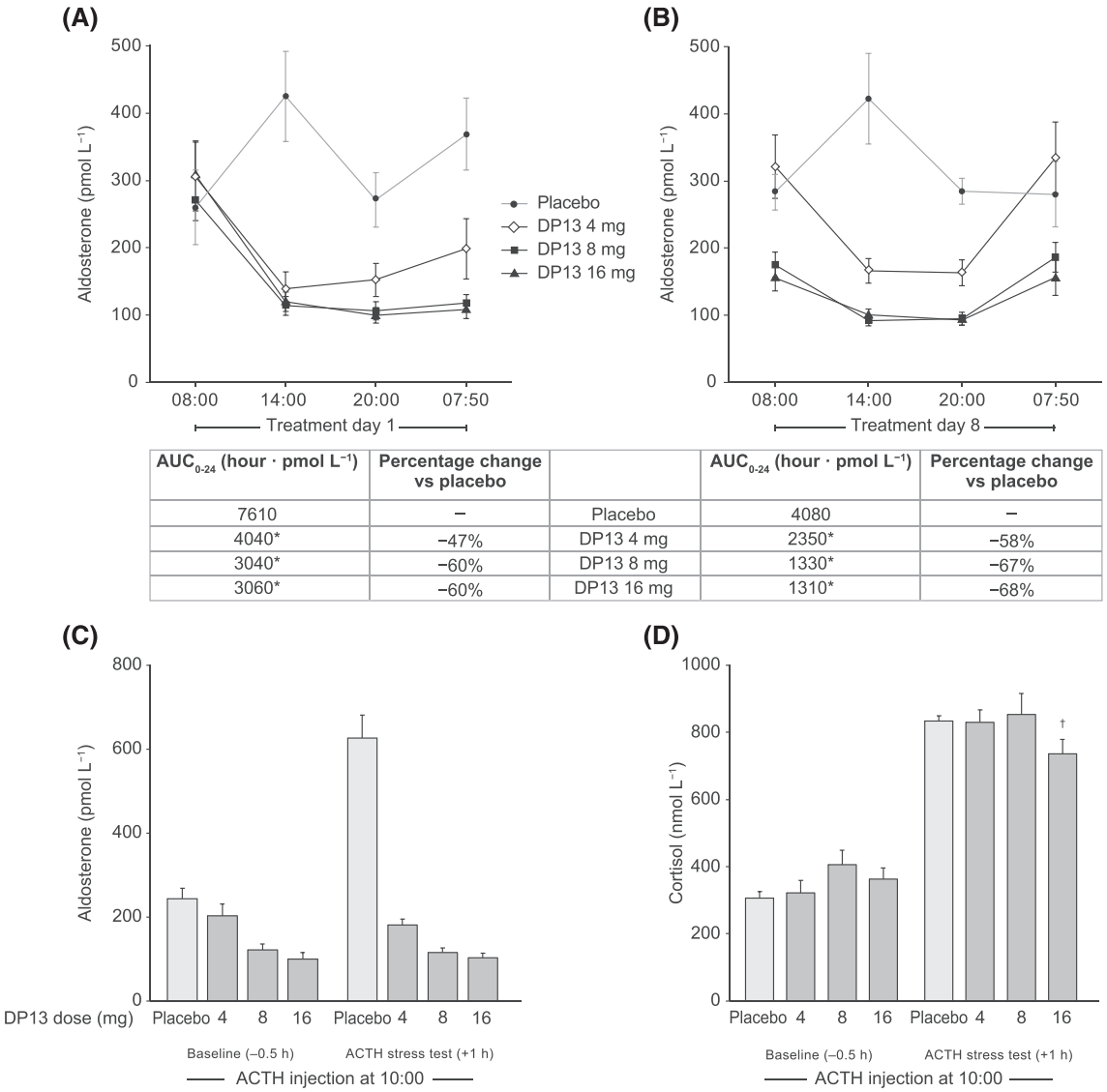
There were no serious AEs, and no participants were discontinued from study part B. Overall, 22 of 32 participants (68.8%) reported 39 TEAEs, of which 36 were mild and three were moderate (Table 1).

Eight TEAEs were considered treatment related. Headache ($n = 6$), abdominal discomfort ($n = 5$), nasopharyngitis ($n = 5$) and cough and dizziness ($n = 3$ for both) were the most common TEAEs. There was no association between the number of TEAEs and DP13 dose or placebo. A blinded central review of Holter-extracted ECG data from the DP13 16 mg dose groups in both study parts A and B identified no clinically significant, treatment-emergent ECG abnormalities. Blood pressure, measured daily at each dose, remained stable (Table S2).

On Days 1 and 8, all three doses of DP13 significantly and dose-dependently suppressed plasma aldosterone compared with placebo as assessed by AUC_{0-24} (geometric mean on Day 8, 1310–2350 *vs.* 4080 pmol L^{-1}) (Figure 3A). In participants receiving DP13 8 mg or 16 mg QD, aldosterone concentration was significantly lower on Day 8 than in those receiving placebo throughout the 24-h interval. In contrast, on Day 8, pre-dose and 24-h post-dose aldosterone levels in participants receiving DP13 4 mg QD were not significantly different from placebo 20 h post-dose (Figure 3B).

In participants receiving placebo, the ACTH stress test performed on Day 7 induced an increase in both serum aldosterone and cortisol compared with baseline (aldosterone, 244 pmol L^{-1} *vs.* 626 pmol L^{-1}; cortisol, 307 nmol L^{-1} *vs.* 833 nmol L^{-1}; Figure 3C,D), whereas in participants receiving DP13 at any dose, aldosterone production was suppressed. Cortisol production for participants receiving DP13 4 or 8 mg QD was similar to those receiving placebo. However, cortisol production was lower in those receiving DP13 16 mg QD than those receiving placebo (736 *vs.* 833 nmol L^{-1}, $P < 0.05$).

On Day 8, post-dose DOC and corticosterone concentrations were higher than baseline values for all doses of DP13



AUC_{0-24} (hour · pmol L^{-1})	Percentage change vs placebo		AUC_{0-24} (hour · pmol L^{-1})	Percentage change vs placebo
7610	–	Placebo	4080	–
4040*	−47%	DP13 4 mg	2350*	−58%
3040*	−60%	DP13 8 mg	1330*	−67%
3060*	−60%	DP13 16 mg	1310*	−68%

FIGURE 3 Plasma aldosterone and cortisol during study part B. plasma concentration of aldosterone on Day 1 (A) and Day 8 (B). Change in ACTH-stimulated aldosterone (C) and cortisol (D) production on Day 7 after daily intake of DP13 or placebo capsules presented as mean and SEM. *$P < 0.05$ *vs.* placebo, †$P < 0.01$ *vs.* placebo. ACTH, adrenocorticotropic hormone; AUC_{0-24}, area under the concentration–time curve from time 0 to 24 h; DP13, dexfadrostat phosphate; SEM, standard error of the mean.

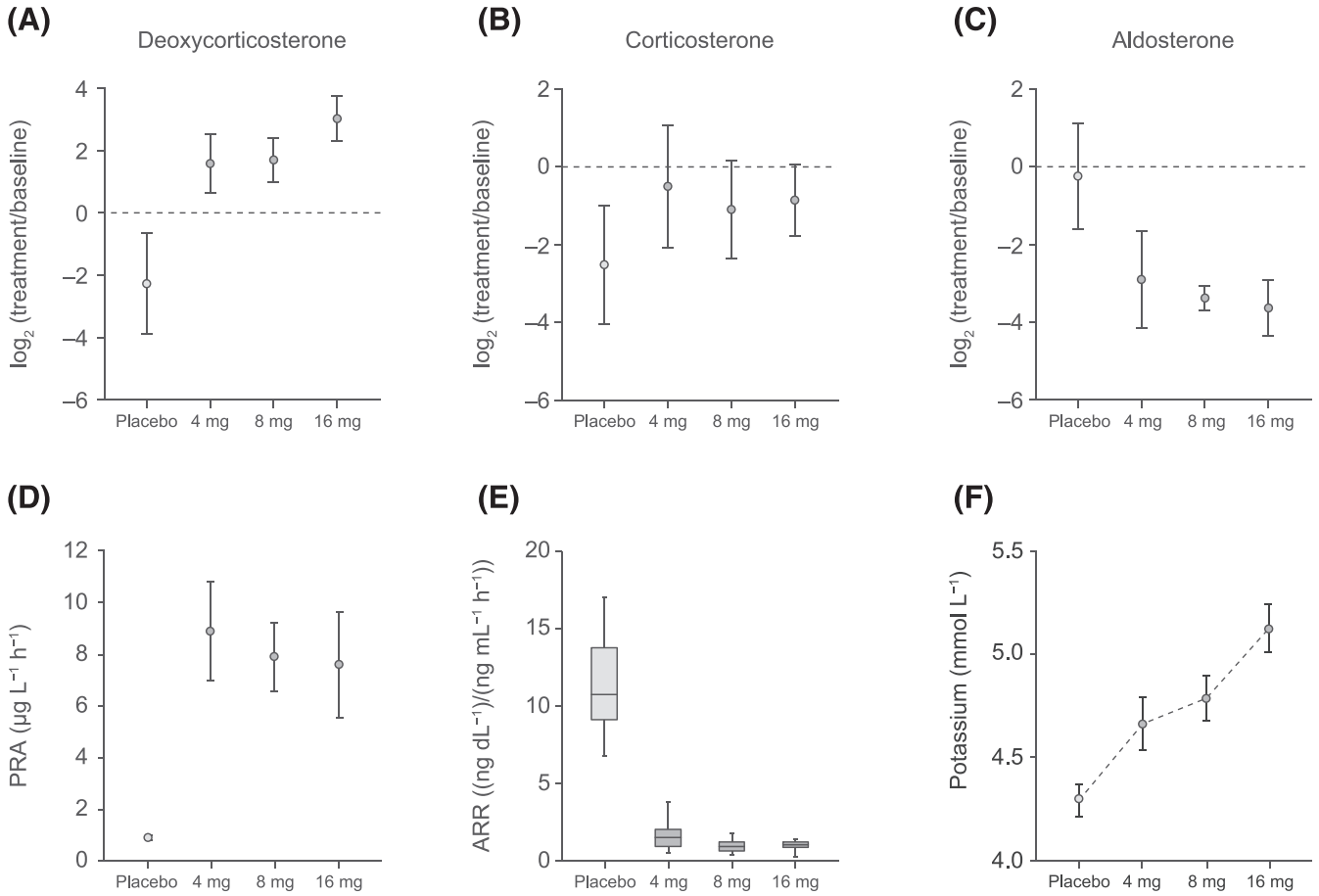


FIGURE 4 Serum aldosterone, aldosterone precursors and renin activity during study part B. Change in blood levels of deoxycorticosterone (A), corticosterone (B) and aldosterone (C) from Day 1 baseline to 12-h post-dose on Day 8. Changes in PRA (D), calculated ARR (E) and blood potassium (F) 24-h post-dose on Day 8. ARR calculated as the ratio of plasma aldosterone concentration (ng dL^{-1}) divided by plasma renin activity (ng mL^{-1} h^{-1}). ARR, aldosterone-to-renin ratio; PRA, plasma renin activity.

(Figure 4A,B). Aldosterone decreased (Figure 4C), while plasma renin activity was increased for all DP13 doses compared with placebo (Figure 4D); accordingly, the calculated aldosterone-to-renin ratio (ARR) was lower in participants receiving DP13 at any dose than those receiving placebo ($P < 0.001$, Figure 4E). Analysis of blood electrolytes 24 h post-dose on Day 8 revealed a dose-dependent increase in potassium concentration (Figure 4F), while there was a dose-independent decrease in sodium concentration in participants receiving any dose of DP13 compared with placebo (Figure S2).

During the pretreatment phase, participants' urinary sodium/potassium ratio was stable. Participants receiving DP13 exhibited a dose-dependent increase in the 24-h urinary sodium/potassium ratio post-dose on Day 1 compared with baseline (Figure 5A–C), while the urinary sodium/potassium ratio on Day 8 was similar to baseline. This was reflected in the urinary sodium concentration, which increased on Day 1 compared with baseline but was similar to baseline levels on Day 8 (Figure 5D–F).

No treatment- or dose-dependent effects on cortisol metabolites or oestradiol were detected in participants receiving DP13 or placebo when comparing baseline and 24-h urine samples on Day 8 (Figure 6A,C). There were also no treatment- or dose-dependent effects on ACTH, LH or FSH measured in pre-dose blood samples on Day 8 compared with baseline (Figure 6B,D,F). Participants receiving DP13 16 mg QD exhibited a reduction in urinary testosterone compared with baseline levels, whereas for participants receiving placebo, DP13 4 or 8 mg QD urinary testosterone levels at Day 8 were similar to those at baseline (Figure 6E). Plasma testosterone concentration did not significantly change with DP13 treatment (Table S3).

The detailed pre- and post-dose plasma DP13 concentrations on Days 1 and 8 and pre-dose trough levels (C_{min}) throughout the multiple QD oral dosing are shown in Figure 7. The PK was similar to that observed in study part A. Examination of the morning C_{min} levels showed that steady state was achieved after three daily doses. No marked accumulation of drug was observed over the entire treatment period. The AUC_{0-t} indicated a small geometric mean accumulation ratio ranging from 1.15 to 1.21 across all dose levels. The geometric mean linearity ratio of systemic exposure to DP13 on Day 8 was between 0.98 and 1.02 (Table S4).

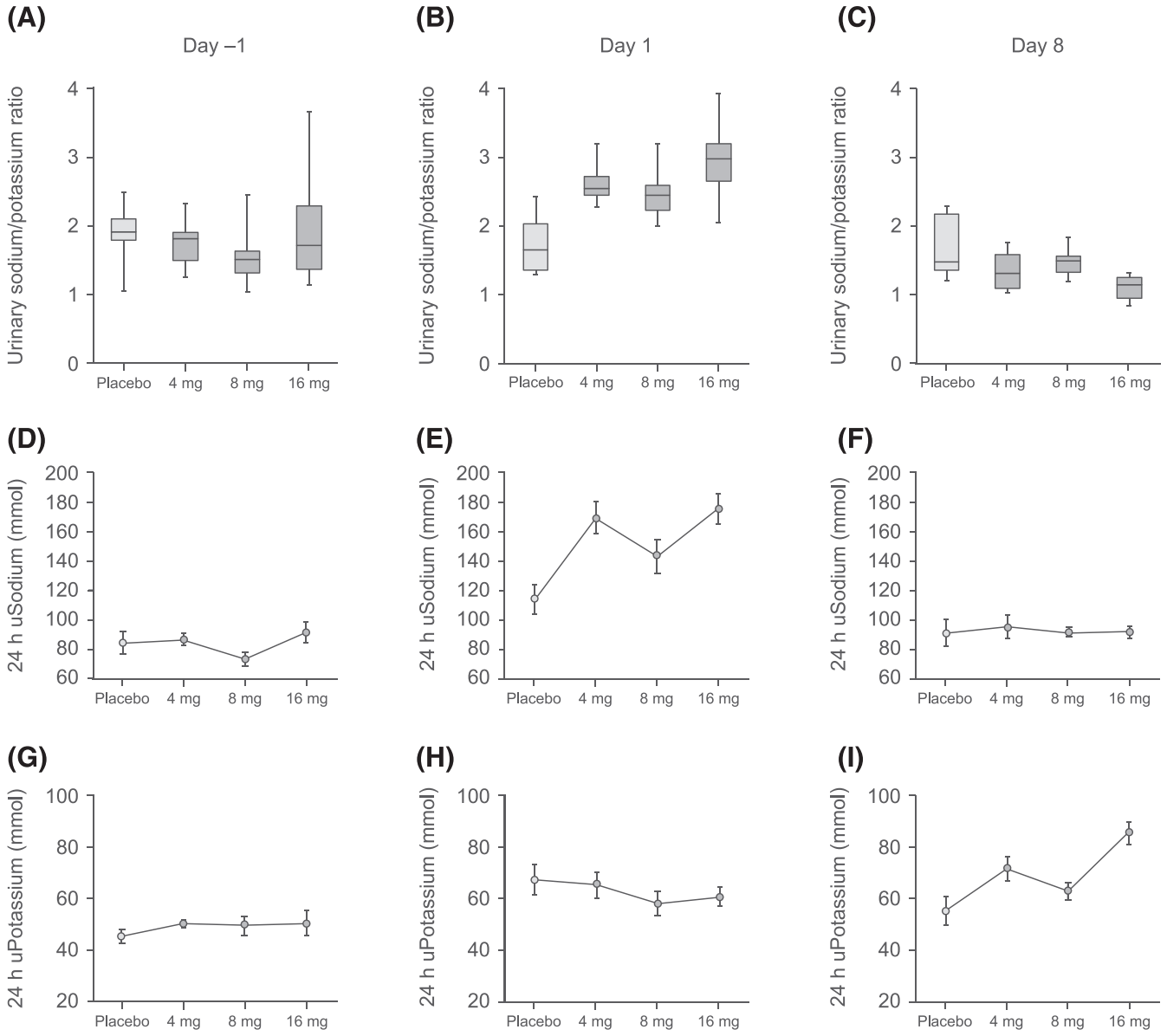


FIGURE 5 Urinary sodium/potassium ratio and individual ion concentrations in 24-h urine collected on Day −1 and on treatment Days 1 and 8 after capsule intake (study part B). Data depicted as interquartile range (box) and minimal–maximal values (whiskers) for urinary sodium/potassium ratio and as mean ± standard error of the mean for individual ion concentrations. uPotassium, urinary potassium; uSodium, urinary sodium.

4 | DISCUSSION

Direct inhibition of CYP11B2, the rate-limiting enzyme for aldosterone synthesis,[8,9] may offer an effective treatment option for aldosterone-dependent hypertension and PA. By acting as a pharmacological adrenalectomy, a CYP11B2 inhibitor could treat a broad patient population. Moreover, CYP11B2 inhibition offers several advantages over currently available treatments for PA: effectiveness in both unilateral and bilateral PA, avoidance of off-target side-effects associated with mineralocorticoid receptor antagonists (menstrual dysfunction and gynaecomastia)[22] and the ability to monitor treatment response via a patient's ARR.

However, CYP11B2 inhibition presents several challenges, most of which are a consequence of the potential to interfere with other CYP450 enzymes involved with steroidogenesis. Any potential CYP11B2 inhibitor must minimally impact the stress response or other steroid hormones. Particularly challenging is to avoid unduly inhibiting CYP11B1 that converts **11-deoxycortisol** to cortisol and shares 93% homology with CYP11B2.[23] Moreover, it is important to avoid excessive accumulation of the aldosterone precursor, DOC, because like aldosterone, it is a mineralocorticoid but whose activity, unlike aldosterone, is tempered by its binding to corticoid binding protein (CBG).[24]

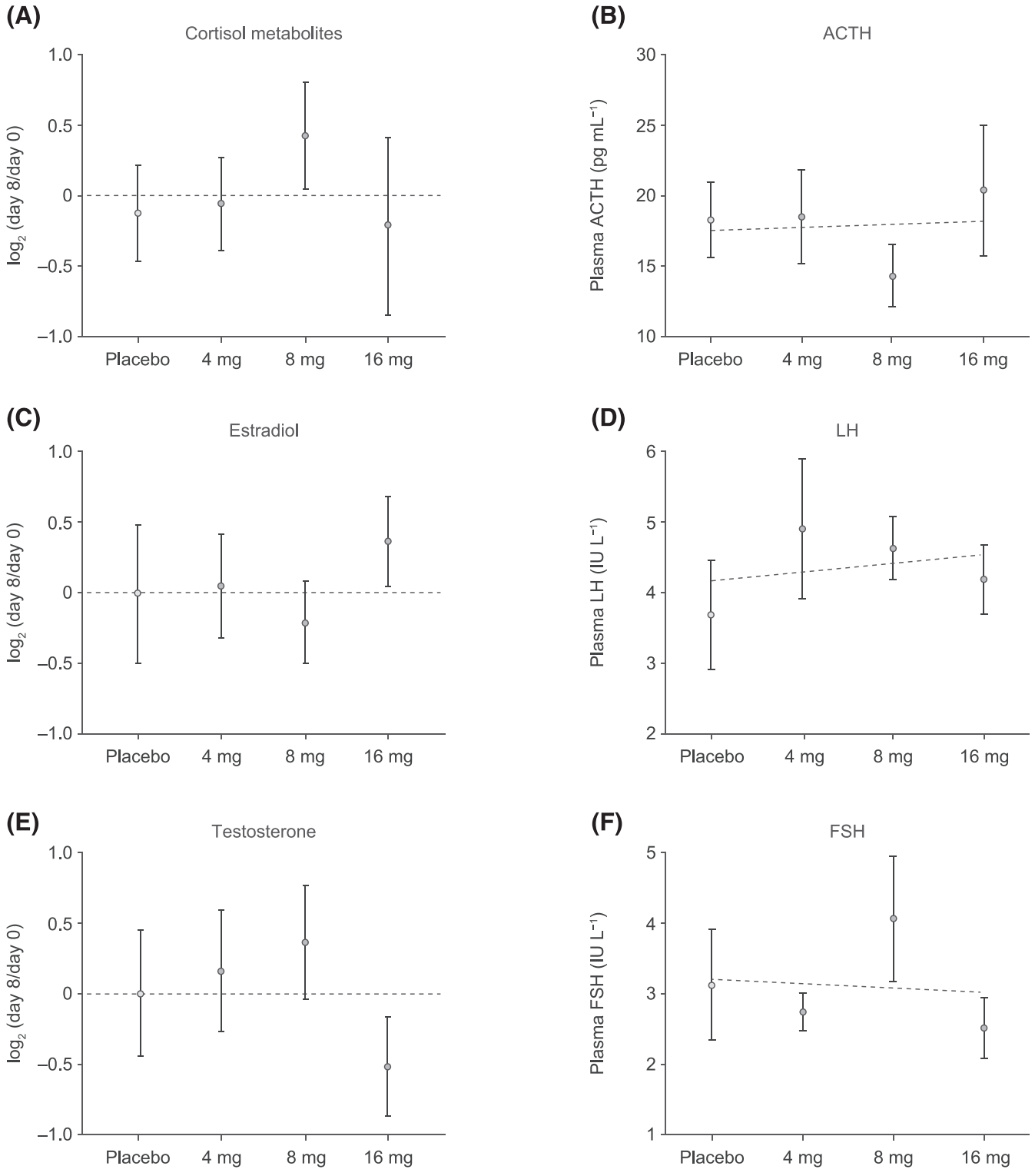


FIGURE 6 Changes in steroid and hormone levels in urine and plasma from baseline to Day 8 (study part B). ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

This first-in-human study evaluated the safety and efficacy of single and multiple doses of the CYP11B2 inhibitor, DP13, in healthy volunteers. In both study parts, there were no serious AEs and no treatment- or dose-related trends in TEAEs, indicating that 1–16 mg of DP13 is well-tolerated.

Periods of maximal dose exposure should demonstrate target selectivity (unimpaired function of the hypothalamic–pituitary–adrenal [HPA] and HPG axes). Maintaining cortisol synthesis is particularly important, because its upregulation by ACTH is critical during exposure to excessive stress, and any significant suppression would induce

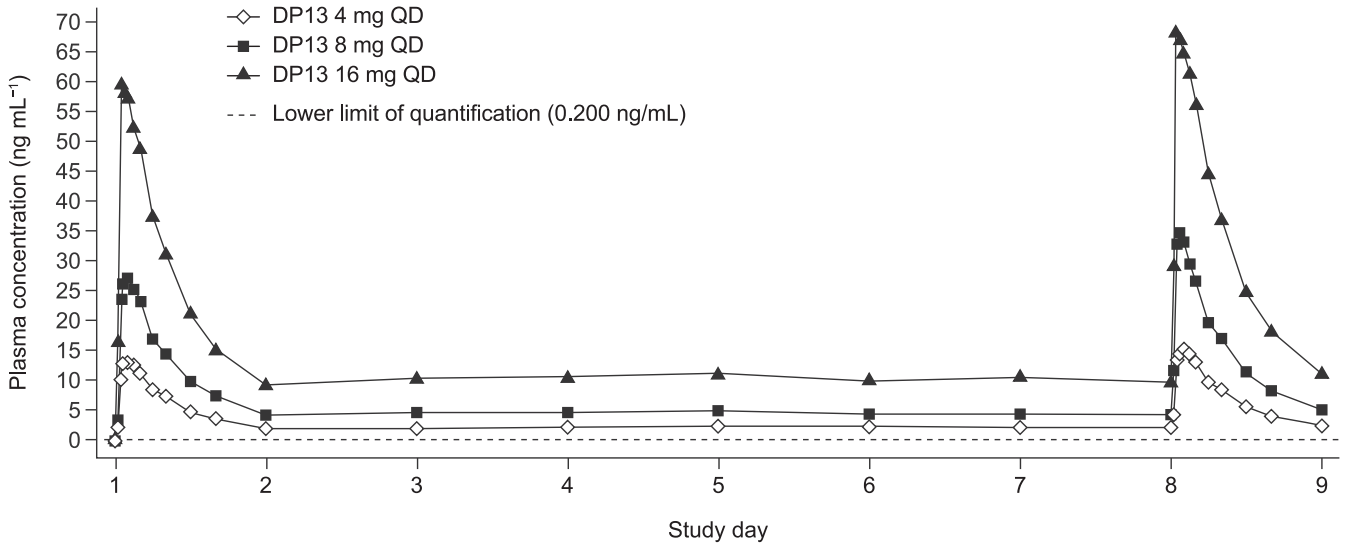


FIGURE 7 Mean plasma concentrations of DP13 during study part B. DP13, dexfadrostat phosphate; QD, once daily.

the HPA feedback loop, resulting in adrenal biosynthesis and potentially hypertrophy.[25] An ACTH stimulation test was used to select DP13 doses at C_{max} that effectively suppressed aldosterone production but not cortisol excursions. Because all tested doses demonstrated no apparent loss of target selectivity, doubling doses from 4 to 8 mg and to 16 mg were selected for the multiple-ascending study part. In that part, the ACTH stimulation test was repeated after 7 days of treatment to confirm aldosterone suppressing activity and unaffected cortisol excursions. DP13 completely blunted any increases in aldosterone that were observed in participants receiving placebo, in whom aldosterone levels reached the low- to mid-range of that observed in patients with PA.[26] This suppression of aldosterone was achieved at all doses and was not accompanied by a blunting of the cortisol response to ACTH, except slightly at the 16 mg dose. Importantly, none of the plasma cortisol concentrations of the DP13-treated individuals fell within the diagnostic range for adrenal insufficiency (<500 nmol L^{-1}).[27] Periods of minimal dose exposure should demonstrate consistent target efficacy, that is, aldosterone suppression that not only addresses diurnal aldosterone production but also can overcome endocrine counter-regulation with increased plasma renin activity and potassium levels. A once daily intake of DP13 in the morning could occur after the diurnal aldosterone peak if the C_{min} steady-state drug levels assure suppression of stimulated morning aldosterone production. The present data indicate that DP13 dosed daily at 4 mg did not maintain aldosterone suppression over 24 h on Day 8, unlike the 8 and 16 mg doses.

Inhibition of CYP11B2 at the first step of aldosterone synthesis results in the accumulation of DOC. When blood levels of DOC far exceed 70 ng dL^{-1} (2.12 nmol L^{-1}), the CBG binding capacity can be exhausted and sodium retention may result.[28] In the current study, levels of DOC were well below the 70 ng dL^{-1} threshold at all doses. Thus, the reduction of mineralocorticoid activity resulting from DP13-mediated suppression of aldosterone production was not compromised by a marked elevation in DOC. It was previously noted by Demers et al. that inhibition of the final step in aldosterone synthesis, the conversion of 18-hydroxycorticosterone to aldosterone by CGS16949A, results in an elevation of the plasma 18-hydroxycorticosterone to aldosterone ratio, as well as an elevation of the urinary metabolites of 18-hydroxycorticosterone relative to the urinary metabolites of aldosterone.[11] This supports the suggestion that CGS16949A blocked the last step in aldosterone synthesis, and blockage at this step would lower aldosterone production without contributing to any further increase in DOC beyond that resulting from the inhibition of the first step (11-β hydroxylase) in the CYP11B2 reaction.

The potential for DP13 to disrupt the HPG axis by inhibition of CYP19A1 activity was addressed by measuring urinary oestradiol and testosterone secretion and plasma LH and FSH after 8 days of treatment. Urinary oestradiol, LH and FSH levels were unaltered across all doses, indicating that DP13 did not inhibit CYP19A1 activity. We observed a decrease in the urinary excretion of testosterone in participants receiving the 16 mg dose compared with placebo; however, this was not accompanied with a change in plasma LH or testosterone levels. In healthy men, LH is negatively regulated by testosterone[29] and would therefore be expected to increase following any clinically significant reduction in testosterone. In a similar study, CGS16949A administered to healthy participants for 14 days resulted in significant suppression of oestradiol and elevations in testosterone and FSH.[30] In males and postmenopausal females, the HPG axis is regulated by oestradiol produced locally in the medial basal hypothalamus/median eminence region and pituitary gland, as well as by androgens produced in the adrenal and gonads.[31–33] Thus, the finding that DP13 did not affect the male HPG axis indicates that it did not significantly alter local CYP19A1 activity in the median eminence or pituitary gland.

The present healthy volunteer study delineated a clear therapeutic window for DP13. The lower range of the window was defined with the 4 mg dose, which resulted in significant change in the sodium/potassium balance and triggered a maximal renin

increase despite incomplete aldosterone suppression over 24 h. The 8 mg dose lowered aldosterone over the entire 24-h dose interval, reducing morning trough levels further. The 16 mg dose of DP13 slightly further reduced blood aldosterone levels but induced a significant elevation of blood potassium levels to the upper range of normokalaemia. Urine sodium concentrations increased from baseline to Day 1 and returned to baseline levels by Day 8; no changes in blood pressure were observed. This is to be expected in participants without hypertension, in whom a compensatory back-shift in pressure natriuresis induced by upregulation of renin and angiotensin II formation leads to a new sodium steady state, as observed with other diuretics. Despite the absence of any dose-dependent changes in basal stress and sex hormone levels or general safety signals, the 16 mg dose may define the upper range of the DP13 therapeutic window.

Given that this phase 1 trial included relatively small numbers of healthy male participants, progression to a phase 2 trial will be required to investigate the efficacy and tolerability of DP13 in larger numbers of patients. Although the ARR reduction is consistent with the proposed therapeutic application of DP13, further studies will be required to determine whether this extends to clinical outcomes such as reduced hypertension and a decreased risk of cardiovascular disease. Aldosterone suppression was assessed from plasma levels at predefined time points. Assessing the urinary concentration of aldosterone and other steroid metabolites may provide further data on the mechanism of CYP11B2 inhibition by DP13 and will be elucidated in future studies.

In this study in healthy volunteers, DP13 distributed dose-dependently and suppressed aldosterone production effectively despite physiological counter-regulation. It remains to be tested how effectively DP13 lowers aldosterone produced in genetically dysregulated, salt suppression test-resistant adrenal glands in patients with PA in comparison with normal adrenal glands in healthy volunteers with a functional endocrine feedback loop. A phase 2 clinical study in patients with PA, to assess the efficacy and safety of DP13 at three doses over an 8-week period, is in progress.

CONTRIBUTORS

All the authors contributed substantially to the conception and design of the manuscript. Ronald Edward Steele, Christoph Schumacher and Hans-Rudolf Brunner were responsible for the conception and design of the experiments; Ashley Brooks, Stuart Hossack and Michael Groessl performed the research; Paolo Mulatero, Ronald Edward Steele, Hans-Rudolf Brunner, Bruno Vogt and Christoph Schumacher were responsible for the analysis and interpretation of the data. All authors were responsible for drafting the article or revising it critically for important intellectual content. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

M.G. is a minority shareholder of Damian Pharma AG. C.S. and R.E.S. are founders and shareholders of Damian Pharma AG. A.B. and S.H. are employees of Covance. P.M., B.V. and H.R.B. declare no conflict of interest with the contents of this article.

DATA AVAILABILITY STATEMENT

The authors will not make data collected for the study available to others, including individual participant data, owing to privacy and ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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