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ARTICLE

Safety, Tolerability, and Pharmacokinetics of the Mineralocorticoid Receptor Modulator AZD9977 in Healthy Men: A Phase I Multiple Ascending Dose Study

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Excessive activation of the mineralocorticoid receptor (MR) underlies the pathophysiology of heart failure and chronic kidney disease. Hyperkalemia risk limits the therapeutic use of conventional MR antagonists. AZD9977 is a nonsteroidal, selective MR modulator that may protect nonepithelial tissues without disturbing electrolyte balance. This phase I study investigated the safety, tolerability, pharmacokinetics, and pharmacodynamics of multiple oral doses of AZD9977 in healthy volunteers. Twenty-seven male participants aged 23–45 years were randomized 3:1 to receive oral AZD9977 or placebo for 8 days (with twice-daily dosing on days 2–7), in dose cohorts of 50, 150, and 300 mg (AZD9977, *n* = 6 per cohort; placebo, *n* = 3 per cohort). Adverse events occurred in 4 of 18 participants receiving AZD9977 (22.2%) and 6 of 9 receiving placebo (66.7%), all of mild or moderate severity; none were serious or led to withdrawal. AZD9977 was rapidly absorbed, with median time of maximum concentration of 0.50–0.84 hours across dose groups. Area under the curve and maximum concentration were approximately dose proportional but elimination and accumulation terminal half-life increased with dose. Steady-state was reached after 3–4 days, with dose-dependent accumulation of 1.2–1.7-fold. Renal clearance was 5.9–6.5 L/hour and 24–37% of AZD9977 was excreted in the urine. Serum aldosterone levels increased dose dependently from days –1 to 7 in participants receiving AZD9977, but serum potassium levels and urinary electrolyte excretion were unchanged. AZD9977 was generally well-tolerated with no safety concerns. Exploratory outcomes suggested reduced hyperkalemia risk compared with MR antagonists. These findings support further clinical development of AZD9977.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Excessive activation of the mineralocorticoid receptor (MR) has a central role in the pathophysiology of heart failure and chronic kidney disease. The risk of hyperkalemia limits the therapeutic use of conventional MR antagonists in patients with these diseases. AZD9977 is a nonsteroidal, selective MR modulator that may protect nonepithelial tissues from damage without disturbing electrolyte balance.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This was a phase I study investigating the safety, tolerability, pharmacokinetics, and pharmacodynamics of multiple oral doses of AZD9977 in healthy subjects.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ No clinically relevant safety and tolerability findings were observed. AZD9977 was rapidly absorbed and had an accumulation half-life of 4–9 hours.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Target engagement shown by a dose-dependent elevation in serum aldosterone levels, with no significant effects on either serum potassium levels or urinary sodium excretion in healthy subjects. The results support further clinical development of AZD9977 in patients to show reduced hyperkalemia risk compared with approved MR antagonists.

Heart failure affects ~ 1–2% of the global population,[1] and chronic kidney disease (CKD) occurs in ~ 11–15%.[2] The prevalence of both these diseases is increasing with the global epidemic of obesity, diabetes, hypertension, and an aging population.[3,4] Approximately half of all patients with heart failure have heart failure with preserved ejection fraction (HFpEF), and there are no treatments available that have conclusively been shown to reduce mortality or to

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improve quality of life in these patients.[5] Furthermore, renal dysfunction is common in patients with HFpEF, and CKD is associated with an increased risk of cardiovascular events; in turn, heart failure is a risk factor for developing CKD.[6]

Excessive mineralocorticoid receptor (MR) activation is a central factor in the pathophysiology of both heart failure and CKD.[7,8] The endogenous agonists aldosterone and cortisol activate the MR,[7] a member of the nuclear receptor subfamily, which is expressed in the cardiovascular system, the kidneys, and the central nervous system, as well as in adipose and other tissues.[8] In nonepithelial tissues, MR activation promotes inflammation, oxidative stress, fibrosis, and endothelial dysfunction, leading to cardiovascular and renal injury.[7,8] Physiologically, the MR plays a central role in regulation of blood pressure as well as fluid and electrolyte balance.[9,10] In kidney epithelial cells, MR activation increases sodium resorption in the renal tubule, which results in concomitant water retention and potassium secretion.[9,13]

The MR antagonists, spironolactone and eplerenone, are approved for the treatment of heart failure with reduced ejection fraction and have been shown to reduce mortality.[5] Spironolactone and eplerenone may improve outcomes in patients with HFpEF, but efficacy results from clinical trials have generally been inconclusive.[14,15] The most convincing evidence in support of the use of MR antagonists in HFpEF comes from a *post hoc* analysis of the phase III Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist (TOPCAT) trial, conducted in 3,445 patients with HFpEF with a mean follow-up of 3.3 years.[14] Although spironolactone had no significant effect on the primary end point (a composite of death from cardiovascular causes, aborted cardiac arrest, or hospitalization for heart failure) in the TOPCAT trial,[14] a *post hoc* analysis revealed regional differences in patient characteristics, adherence to study drugs, and responses to spironolactone, as well as a profound difference in event rates for patients in the United States, Canada, Brazil, and Argentina vs. those in Russia and Georgia.[17] Analysis of the patients recruited from the Americas suggested that spironolactone treatment may improve clinical outcomes in HFpEF, and this analysis warrants further investigation.[17] In patients with CKD, MR antagonists have been shown to reduce proteinuria and may improve kidney function.[18] They may also prevent the development of cardiovascular disease and progression to end-stage renal disease in patients with CKD.[19] Despite the demonstrated and potential efficacy of MR antagonists in these indications, their mechanism of action results in potassium retention, leading to a risk of hyperkalemia. This safety profile limits their use in treating patients with heart failure (especially those with comorbid kidney disease) and patients with CKD.[20,21]

AZD9977 is a nonsteroidal, selective MR modulator that blocks the damaging action of aldosterone and cortisol in nonepithelial tissues, but has minimal effects on electrolyte handling by kidney epithelial cells.[25] This tissue-specific modulation means AZD9977 could provide protection against the progression of cardiovascular and renal disease in patients with HFpEF, CKD, or both diseases, without the risk of hyperkalemia associated with steroidal MR antagonists, such as spironolactone and eplerenone. The binding of AZD9977 to the MR induces a unique protein conformation in the MR that is distinct from the one induced by MR antagonists, and may modify the interaction pattern of the receptor with transcriptional co-activators.[25] In a first-in-human phase I study, single doses of AZD9977 covering the range 5–1,200 mg had a good safety profile and were well-tolerated in healthy volunteers.[26] AZD9977 was rapidly absorbed after oral administration, with dose-proportional systemic exposure up to 200 mg and a short plasma half-life ($t_{1/2}$) of 2–3 hours. The human plasma protein binding of AZD9977 is 82%, and *in vitro* metabolism studies have shown that the cytochrome P450 (CYP) enzymes CYP3A4 and CYP3A5 are the main CYP isoforms involved in the metabolism of AZD9977. The permeability of AZD9977 in Caco-2 cells at pH 6.5 is 5.2×10^{-6} cm/second with an efflux ratio of 10.5 and a solubility of 674 μM in the same system (unpublished data). Here, we describe a phase I study of multiple oral doses of AZD9977 in healthy volunteers. The study aimed to investigate the safety, tolerability, and pharmacokinetics of AZD9977, as well as its pharmacodynamic effects on aldosterone and electrolyte levels.

METHODS

Conduct and ethics

An independent ethics committee (National Research Ethics Service South Central – Berkshire B, UK) and regulatory authority (Medicines and Healthcare Products Regulatory Agency, UK) reviewed and approved the study protocol and its amendments. The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Council for Harmonisation Good Clinical Practice guidelines,[27] and is registered on ClinicalTrials.gov (identifier: NCT03435276). All participants gave freely their written informed consent before starting the study. The study took place from February to June 2018 at the PAREXEL Early Phase Clinical Unit in Northwick Park Hospital, London, UK.

Overview and objectives

This was a randomized, placebo-controlled, single-blind phase I study of the safety, tolerability, and pharmacokinetics of AZD9977 in sequential ascending dose groups of healthy men receiving oral AZD9977 for 8 days. Participants were blinded to treatment assignment and study site staff were blinded during clinical conduct of each cohort. The primary objective of the study was to investigate the safety and tolerability of AZD9977 after multiple dosing. The secondary objectives were to characterize the pharmacokinetics of AZD9977, including assessments of the time needed to reach steady-state plasma levels of the drug, the extent of drug accumulation in the body, and the dependence of pharmacokinetic parameters on time.

Exploratory objectives were to assess the effects of AZD9977 on serum and urinary electrolyte levels, serum aldosterone levels, and plasma 4ß-hydroxycholesterol levels. Monitoring 4ß-hydroxycholesterol levels in the first multiple-dose study in humans is part of the published AstraZeneca strategy for assessing potential CYP3A induction with investigational new drugs.[28] Additionally, an oral

glucose tolerance test (OGTT) was performed on day –1 and day 7, because a small reversible increase in plasma glucose level was observed in preclinical rat toxicology studies (all values within the normal range).

Participants

Male volunteers aged 18–50 years, weighing 50–100 kg, and with a body mass index of 18–30 kg/m^2 were eligible for the study. Key exclusion criteria were: history or presence of any disease or disorder that might influence study participation or results; history or presence of any condition known to interfere with the absorption, distribution, metabolism, or excretion of drugs, including gastrointestinal, hepatic, or renal disease; any clinically significant illness, medical procedure, or trauma within the previous 4 weeks; any clinically important abnormalities in clinical chemistry, hematology, or urinalysis (including serum potassium > 5.0 mmol/L and glycated hemoglobin > 5.7%); abnormal vital signs (including systolic blood pressure < 90 mmHg or > 140 mmHg, diastolic blood pressure < 50 mmHg or > 90 mmHg, and heart rate < 45 or > 85 beats per minute); or any clinically important electrocardiographic abnormalities. Volunteers who tested positive for nicotine, alcohol, or recreational drugs were ineligible, as were those with excessive caffeine intake.

Randomization and blinding

Participants were divided into three sequential ascending dose cohorts (n = 9 per cohort). Within each cohort, participants were randomized to receive AZD9977 (n = 6) or placebo (n = 3). This was a single-blind study in which study center staff were also blinded to treatment assignment during the clinical conduct of each cohort. AZD9977 and placebo matched in formulation, appearance, and administration volume. Safety data from each sequential dose cohort were reviewed unblinded. Treatment assignment could be revealed if necessary in a medical emergency.

Study drug administration

In each cohort of nine participants, dosing started with two sentinel participants (AZD9977, n = 1; and placebo, n = 1) and continued in the remaining participants after review of safety data from the sentinel participants at 72 hours after their first dose. Participants resided at the study center from 2 days before their first dose until at least 36 hours after their last dose and attended a follow-up visit 5–7 days after their last dose. Screening took place within 28 days of admission.

In the first cohort, each participant received oral AZD9977 50 mg or matching placebo from day 1 to day 8, with single doses on day 1 and day 8 and twice-daily dosing on days 2–7 (a total of 14 doses). Doses were escalated to AZD9977 150 and 300 mg in the subsequent cohorts after review of safety and pharmacokinetic data from previous cohorts. The starting dose was based on preclinical pharmacokinetic–pharmacodynamic modeling data and on the previous observation that single doses of AZD9977 up to 1,200 mg were well-tolerated in healthy men.[26] AZD9977 was administered as a 15 mg/mL oral suspension in a volume of 240 mL.

On day 1 and day 8, participants fasted for 10 hours before morning dosing and until 4 hours after dosing, when lunch was served. On days 2–7, participants fasted for 10 hours before morning dosing and until 1 hour after morning dosing, when breakfast was served; and from 2 hours before evening dosing until 1 hour after evening dosing. Participants could drink freely except during the 1 hour before and after dosing. No concomitant medication was allowed during the study other than paracetamol (acetaminophen), including herbal remedies, vitamin supplements, and over-the-counter products. While resident at the study center, participants received a standard diet that excluded alcohol and products containing grapefruit. Participants refrained from lying fully supine for 4 hours after dosing (unless required for study assessments) and refrained from strenuous activity from 72 hours before admission to the study center until follow-up.

Safety assessments

Safety was assessed throughout the study by monitoring adverse events, cardiac telemetry, vital signs, and electrocardiographic parameters, conducting physical examinations and laboratory analyses, and collecting urine to measure electrolyte excretion. Criteria for stopping the study on the grounds of safety were predefined. The safety analysis set included all participants who received at least one dose of AZD9977 or placebo.

Pharmacokinetic analyses

Plasma was collected for pharmacokinetic analyses before all doses and at 20 minutes, 40 minutes, and 1, 2, 4, 6, 8, 10, 12, 16, and 20 hours after dosing on day 1 and day 8, then at 24, 30, and 36 hours after the final dose. Urine samples were collected on day 8 (before and 0–4, 4–8, and 8–12 hours after dosing).

Plasma pharmacokinetic outcomes included the observed maximum concentration (C_{max}), time to C_{max} (T_{max}), $t_{1/2}$ associated with terminal slope (λz) of a semilogarithmic concentration–time curve ($t_{1/2\lambda z}$), $t_{1/2}$ based on drug accumulation at steady state ($t_{1/2acc}$), area under the concentration–time curve extrapolated to infinity ($AUC_{0-\infty}$) and in the dosing period (AUC_{τ}), apparent clearance (Cl/F), renal clearance (Cl_R), and accumulation ratio (R_{ac}). Actual blood sampling times were used in all analyses and plasma concentration over time data were analyzed by noncompartmental analysis with Phoenix WinNonlin (version 6.2 Pharsight, Mountain View, CA). The pharmacokinetic analysis set included all participants in the safety set with calculable pharmacokinetic data for at least two treatment periods and without protocol deviations that might have affected pharmacokinetic data.

Exploratory outcomes

Glucose homeostasis was assessed by OGTT on day –1 and immediately after dosing on day 7. Blood samples for analysis of glucose, insulin, and C-peptide were taken before and 2 hours after the administration of oral glucose at the same time of day on the 2 test days. Participants fasted overnight before testing and continued to fast until testing was complete.

Blood samples were taken for measurement of electrolyte and aldosterone levels immediately before administration of

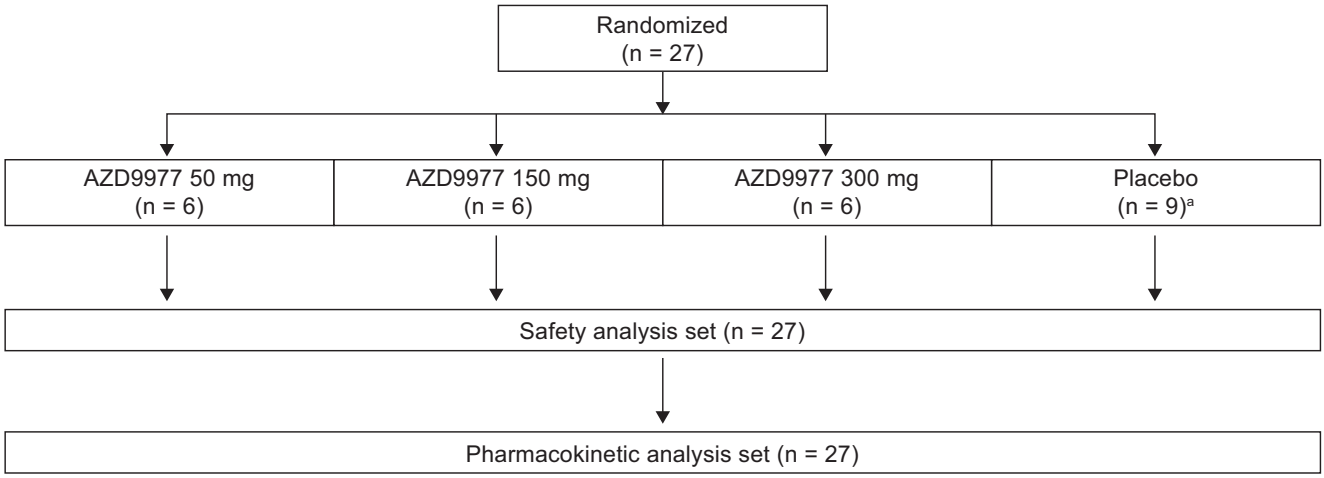

Figure 1 Participant disposition. Indicated doses were taken once daily on day 1 and day 8, and twice daily on days 2–7. [a]*n* = 3 per cohort.

oral glucose on day –1 and day 7. Urinary electrolytes were assessed for 12 hours after administration of oral glucose on day –1 and day 7, using urine collection intervals of 0–3, 3–6, 6–9, and 9–12 hours after administration.

Induction of CYP450 3A4/3A5 was assessed by sampling blood for measurement of 4ß-hydroxycholesterol levels before dosing on day 1 and day 8.

Bioanalysis of blood and urine samples

Urine and plasma samples for analysis of AZD9977 and 4ß-hydroxycholesterol concentrations were stored at –20°C until analysis within the known stability period. Samples were analyzed by Covance Laboratories (Harrogate, UK). AZD9977 and the stable-labeled internal standard [$^{13}CD_3$]AZD9977 were extracted from plasma by protein precipitation and from urine by sample dilution, before analysis by liquid chromatography and tandem mass spectrometry. The methods were validated in the range 10–10,000 nmol/L before sample analysis. Inter-run accuracy and precision were in the ranges of 99.0–100.6% and 1.3–7.2% for plasma and 99.5–100.8% and 1.1–6.7% for urine, respectively, at concentrations of 10, 30, 500, 8,000, and 25,000 nmol/L in plasma and urine. Each analytical run included a calibration curve, appropriate blanks, and duplicate quality control samples at three concentrations within the calibration range. During the study, incurred sample reproducibility analyses showed that 72 of 72 plasma (100%) and 20 of 20 urine (100%) samples tested were within 20% of the mean of the two values, which fulfilled criteria for acceptable performance. Levels of 4β-hydroxycholesterol in plasma were analyzed as previously reported.[29] Other biomarkers were assayed using standard clinical laboratory methods.

Statistical methods

This sample size was chosen based on previous experience of phase I studies to obtain reasonable evidence of safety and tolerability without unnecessary exposure of healthy volunteers to the investigational drug. Sample size was not based on statistical power calculation.

RESULTS

Participant disposition and demographics

All 27 randomized participants completed the study and were included in the safety and pharmacokinetic analysis sets (**Figure 1**). The 18 participants randomized to AZD9977 received doses of 50, 150, or 300 mg with twice-daily dosing on days 2–7 (six per cohort) and the remaining nine participants received placebo (three per cohort). All participants were men aged 23–45 years and most were white (**Table 1**).

Safety and tolerability

Multiple ascending oral doses of AZD9977 up to 300 mg twice daily were well-tolerated and no safety concerns were raised. There were no serious adverse events or deaths. Seven adverse events occurred in 4 of the 18 participants (22.2%) receiving AZD9977, and eight events occurred in six of the nine participants (66.7%) receiving placebo. All adverse events were of mild or moderate intensity, and none resulted in withdrawal from the study. In the AZD9977 groups, three adverse events were reported in the 50 mg group (headache, two events; cellulitis, one event), two in the 150 mg group (back pain, one event; dizziness, one event), and two in the 300 mg group (musculoskeletal chest pain, one event; ear discomfort, one event). In the placebo group, the adverse events reported were headache (three events), swelling (one event), seasonal allergy (one event), nasal congestion (one event), and contact dermatitis (two events). Only the headache events were considered related to the study drug by the investigator. No clinically meaningful changes in blood pressure, heart rate, or laboratory values were observed with AZD9977 (including serum sodium, potassium, bicarbonate, and calcium levels).

Pharmacokinetics

AZD9977 was rapidly absorbed after oral administration, with a median T_{max} in the range 0.50–0.84 hours on both day 1 (**Figure 2a**) and day 8 (**Figure 2b**) across all dose groups (**Table 2**). The $t_{1/2\lambda z}$ of AZD9977 increased with dose, from a mean of 4.44 hours in the 50 mg group to 9.62 hours in the 300 mg group on day 8 (**Table 2**). The

Table 1 Participant demographics

Characteristic	AZD9977 50 mg (*n* = 6)	AZD9977 150 mg (*n* = 6)	AZD9977 300 mg (*n* = 6)	AZD9977 total (*n* = 18)	Placebo (*n* = 9)
Age, years					
Mean (SD)	38.5 (4.4)	34.2 (6.2)	28.2 (5.1)	33.6 (6.6)	37.1 (6.6)
Median (range)	38.5 (32–45)	37.0 (23–39)	26.0 (24–38)	36.5 (23–45)	40.0 (24–43)
Height, cm					
Mean (SD)	178.7 (3.2)	176.0 (4.9)	177.3 (4.6)	177.3 (4.2)	176.1 (5.4)
Weight, kg					
Mean (SD)	78.12 (9.36)	78.73 (11.95)	74.13 (5.52)	76.99 (9.01)	81.84 (9.54)
BMI, kg/m^2					
Mean (SD)	24.48 (2.94)	25.32 (2.63)	23.58 (1.53)	24.46 (2.41)	26.37 (2.67)
Race					
White, *n* (%)	6 (100)	5 (83.3)	5 (83.3)	16 (88.9)	7 (77.8)
Other, *n* (%)	0	1 (16.7)	1 (16.7)	2 (11.1)	2 (22.2)

BMI, body mass index.

percentage of the AZD9977 dose excreted in the urine ranged from 2437%, with renal clearance in the range 5.89–6.48 L/hour across doses.

The AUCτ and C_{max} of AZD9977 were approximately proportional to dose on day 8, with modeled slope estimates of 0.871 (90% confidence interval, 0.624–1.12) and 1.04 (0.822–1.26), respectively. Steady-state was reached within 3–4 days of twice-daily dosing with AZD9977, with dose-dependent accumulation of 1.2-fold to 1.7-fold across the dose range (R_{ac} and $t_{1/2acc}$; **Table 2**) and higher trough concentration (C_{trough}) values in the morning compared with the evening (**Figure 3**).

Figure S1 shows AZD9977 concentration–time profiles in individual participants.

Exploratory outcomes

Aldosterone and electrolyte levels. Serum aldosterone levels increased dose dependently from day –1 to day 7 in participants receiving AZD9977, with no change in the placebo group (**Figure 4a**; **Table S1**). Serum potassium levels did not undergo any clinically meaningful changes throughout the study (**Figure 4b**; **Figure S2**). Urinary electrolyte excretion was not affected in any AZD9977 dose cohort, with similar log ratios of sodium to potassium levels for participants receiving AZD9977 and placebo under fasted conditions on day 7 (**Figure 5**). Electrolyte ratios were also similar to those observed on day –1 under uncontrolled feeding conditions (**Figure 5**).

Glucose homeostasis. AZD9977 doses up to 300 mg twice daily had no effect on glucose homeostasis based on the OGTT (data not shown).

CYP450 3A4/3A5 induction. No increases in 4β-hydroxycholesterol levels were observed, with mean changes from before dosing on day 1 to day 7 of –3.28 ng/mL (SD, 1.99), –4.98 ng/mL (SD, 1.54), and –4.82 ng/mL (SD, 5.14) for AZD9977 doses of 50, 150, and 300 mg, respectively.

DISCUSSION

In this phase I study, multiple oral doses of AZD9977 up to 300 mg for 8 days, with twice-daily dosing on days 2–7, were

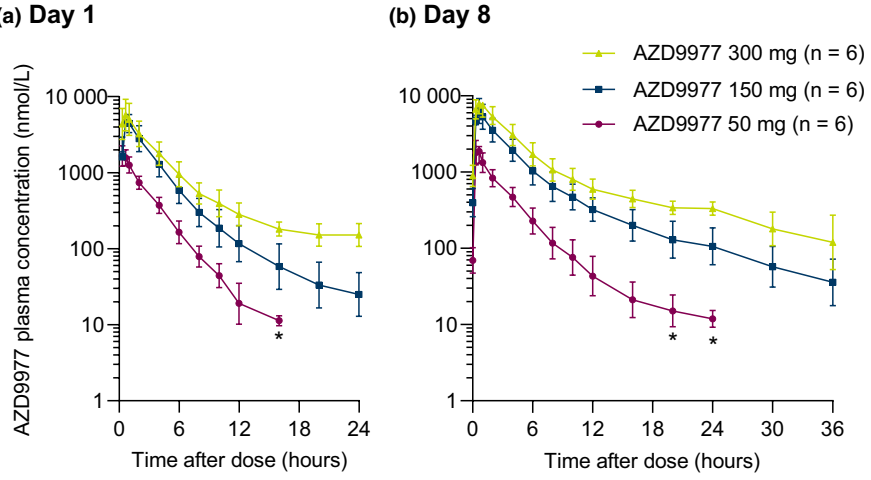

Figure 2 AZD9977 concentration–time profiles on (**a**) day 1 and (**b**) day 8. Data are geometric mean ± geometric SD from the pharmacokinetic analysis set. *Some samples (3) were below the lower limit of quantification (10.0 nmol/L).

Table 2 Pharmacokinetic parameters for AZD9977

	Day 1			Day 8		
Parameter	**50 mg (*n* = 6)**	**150 mg (*n* = 6)**	**300 mg (*n* = 6)**	**50 mg (*n* = 6)**	**150 mg (*n* = 6)**	**300 mg (*n* = 6)**
$AUC_{0-\infty}$, h·nmol/L	4,348 (19.00)	14,600 (32.33)	21,510 (31.20)	NA	NA	NA
AUCτ, h·nmol/L	4,307 (18.71)	13,880 (32.40)	19,150 (35.82)	5,092 (28.73)	20,960 (33.31)	31,600 (30.30)
C_{max}, nmol/L	1,853 (23.80)	4,934 (20.50)	6,242 (42.15)	1,879 (38.00)	6,663 (32.41)	8,506 (32.52)
$t_{1/2\lambda z}$, hour	2.25 (20.37)	5.88 (42.56)	10.62 (33.63)	4.44 (50.53)	8.22 (23.75)	9.62 (66.18)
R_{ac}	NA	NA	NA	1.2 (10.8)	1.5 (12.3)	1.7 (7.3)
$t_{1/2acc}$, hour	NA	NA	NA	4.11 (47.5)	7.53 (25.0)	8.90 (11.8)
Cl/*F*, L/hour	28.5 (18.8)	25.2 (32.2)	27.4 (21.8)	24.6 (28.8)	17.9 (33.3)	23.8 (30.2)
T_{max}, hour	0.50 (0.33–0.66)	0.83 (0.65–2.00)	0.66 (0.33–1.00)	0.50 (0.33–0.66)	0.66 (0.33–0.66)	0.84 (0.31–1.00)
Cl_R, L/hour	NA	NA	NA	5.89 (23.89)	6.48 (21.17)	6.38 (23.52)

Data are geometric mean (coefficient of variation) except for t_{max}, which is median (range). Data are from the pharmacokinetic analysis set.
AUCτ, area under the plasma concentration–time curve in the dosing interval; $AUC_{0-\infty}$, area under the plasma concentration–time curve from time zero extrapolated to infinity; *Cl*/*F*, apparent plasma clearance; Cl_R, renal clearance; C_{max}, observed maximum plasma concentration; NA, not applicable; R_{ac}, accumulation ratio; $t_{1/2\lambda z}$, terminal half-life; $t_{1/2acc}$, accumulation half-life T_{max}, time to reach maximum concentration.

well-tolerated by healthy male volunteers, with no safety or tolerability concerns identified. Plasma levels of AZD9977 reached steady-state after 3–4 days. Following twice-daily dosing, C_{trough} values were higher in the morning than the evening, likely owing to an effect of food intake on absorption. Accumulation and $t_{1/2\lambda z}$ both increased with dose of AZD9977, for reasons that are not fully understood. The limited solubility of AZD9977 and the ongoing absorption of the previous dose in the colon, rather than dose-dependent and/or time-dependent disposition, may explain these findings. Consistent with this possibility, plasma levels of AZD9977 declined more rapidly from ~ 24 to 36 hours after the last dose on day 8 than from 12 to 24 hours after dosing (**Figure 2b**, **Figure S1**). Additional support for this explanation is the similar dose proportionality in exposure on day 1 and day 8, the time needed to reach steady-state, and the stable morning and evening C_{trough} levels at steady-state. Moreover, regional absorption of AZD9977 was explored in a previous study and the extent of colonic absorption was reported to be sufficient for development of a controlled release formulation.[26] AZD9977 did not increase 4β-hydroxycholesterol levels, indicating no induction of CYP450 3A4/3A5.

Serum aldosterone levels increased dose dependently in participants receiving AZD9977, indicating effective target engagement of the MR (**Figure 4a**).[30] Administration of AZD9977 and the associated increases in serum aldosterone levels were not, however, accompanied by any alteration in urinary electrolyte excretion or serum levels of potassium or other electrolytes (**Figure 4b**, **Figure 5**). These results are consistent with a differentiated mechanism of action of AZD9977 when compared with other MR antagonists and are in agreement with the findings in rats fed a low-salt diet, in which AZD9977 also had no effect on the urinary sodium to potassium ratio.[25] The observations made in this study suggest AZD9977 has a unique mode of action supporting early reported findings of MR modulation rather than antagonism. In contrast, with eplerenone,

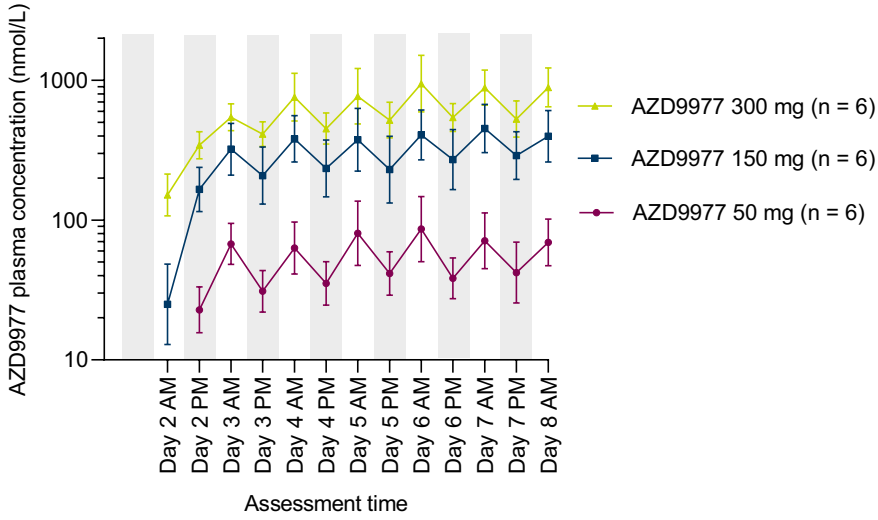

Figure 3 Trough concentration–time profiles after multiple doses of AZD9977 in the morning (AM) and evening (PM). Data are geometric mean ± geometric SD from the pharmacokinetic analysis set. Samples were taken before dosing at 0 hours (AM) and 12 hours (PM). Indicated doses were taken twice daily on days 2–7, and once daily on day 1 and day 8. Shading indicates PM sample.

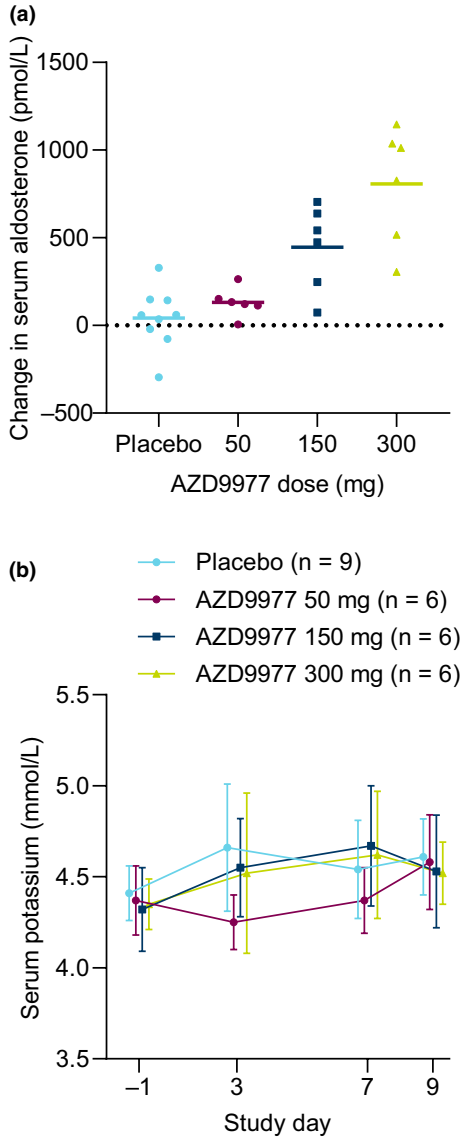

Figure 4 Change in serum aldosterone levels from day –1 to day 7 (**a**) and serum potassium levels throughout the study (**b**). Data on change in serum aldosterone levels show individual participants with the mean for each treatment group and serum potassium levels are mean ± SD, all data from the safety analysis set. Data points are horizontally offset for clarity.

dose-related increases in serum aldosterone levels were accompanied by significant increases in the urinary sodium to potassium ratio after administration of single doses of 100–1000 mg to healthy volunteers. Moreover, transient reductions in serum sodium levels and increases in serum potassium levels were observed after administration of multiple doses of eplerenone 100–1000 mg.[30,31] However, some of these studies were performed under conditions of a low sodium (20 mmol/day) or a restricted sodium (150 mmol/day) and potassium (80 mmol/day) diet, in order to promote distal sodium reabsorption, in contrast to this study in which daily sodium intake was in the normal range.[30,32]

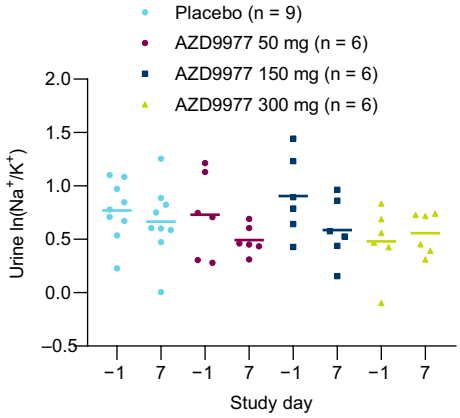

Figure 5 Urine electrolyte levels on day –1 and day 7. Data show the natural logarithm (ln) of the sodium to potassium ratio for individual participants with the mean for each treatment group from the safety analysis set. Analyte levels were the sum of amounts 0–12 hours after administration. Note that participants were on a supervised diet at the clinic on day 7 but not on day –1.

Following fludrocortisone agonist challenge in a previous phase I trial in healthy volunteers, a single dose of AZD9977 unexpectedly increased the urine sodium to potassium ratio to a similar degree to eplerenone.[26] In rats, AZD9977 also increased the urinary sodium to potassium ratio after fludrocortisone challenge, but did not affect the sodium to potassium ratio after aldosterone challenge through either administration of exogenous aldosterone or elevation of endogenous aldosterone levels.[33] In contrast, eplerenone increased the urinary sodium to potassium ratio after both fludrocortisone and exogenous aldosterone challenge in rats.[33] These findings suggest that fludrocortisone affects kidney electrolyte handling differently from aldosterone, possibly because fludrocortisone activates the glucocorticoid receptor as well as the MR at the doses administered in the phase I trial and the rodent studies.[33] Given that AZD9977 is thought to modulate the MR,[25] fludrocortisone challenge in healthy volunteers may not be a suitable model for predicting effects of AZD9977 in patients with aldosterone-mediated conditions, such as heart failure.

The target engagement demonstrated in the present study while potassium levels remained stable in the blood and urine of healthy volunteers is consistent with the potential of AZD9977 to effectively treat patients with cardiac and renal disease, with a reduced risk of hyperkalemia compared with MR antagonists. However, healthy participants may be less prone to hyperkalemia associated with MR modulation than patients with heart failure or CKD, so the effect of AZD9977 on potassium retention needs to be confirmed in target patient populations. A phase I study is ongoing to compare the effect of AZD9977 with that of spironolactone on serum potassium levels in patients with heart failure with preserved or mid-range ejection fraction, and renal impairment (ClinicalTrials.gov identifier: NCT03682497).

The strengths of the current study include the enrollment of the minimum possible number of healthy volunteers to obtain reasonable evidence of safety and tolerability, and the comprehensive safety, pharmacokinetic, and pharmacodynamic assessments conducted. A key limitation of this study

was that potassium retention was not investigated after MR agonist challenge or during elevation of endogenous aldosterone levels, for example, via a low-salt diet. The analyses of urine electrolytes on day –1 and day 7 may have been compromised by the lack of dietary standardization on day –1.

CONCLUSIONS

In this phase I study of AZD9977, no safety or tolerability concerns were identified in healthy volunteers receiving oral doses up to 300 mg for 8 days (with twice-daily dosing on days 2–7). Furthermore, no significant effects on either serum potassium levels or urinary sodium excretion were observed, despite dose-dependent elevations in serum aldosterone levels. The aldosterone response to AZD9977 supports target engagement, and the lack of effect on electrolytes is consistent with its hypothesized mechanism of action. The study confirmed the safety profile and pharmacodynamic effects of AZD9977 observed in a previous single-dose phase I study in healthy volunteers.[26] The results of these studies suggest that AZD9977 may protect against aldosterone-mediated organ damage, with a reduced risk of hyperkalemia compared with MR antagonists. These findings support further clinical development of AZD9977 in patients with HFpEF, as well as those with CKD.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Figure S1. AZD9977 concentration–time profiles for individual participants on day 1 after receiving AZD9977 (**a**) 50 mg, (**b**) 150 mg, and (**c**) 300 mg, and on day 8 after receiving AZD9977 (**d**) 50 mg, (**e**) 150 mg, and (**f**) 300 mg.
Figure S2. Change in serum potassium level from day –1. Data are mean ± SD from the safety analysis set. Data points are horizontally offset for clarity.
Table S1. Serum aldosterone levels.

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Conflict of Interest. A.W., A.M.K., J.H.-G., A.B., P.J.G., M.H., M.K., R.U., L.W., and H.E. are employees of AstraZeneca, and may own stock or stock options. M.A. and P.F. are employees of PAREXEL.

Author Contributions. All authors wrote the manuscript. A.W., A.M.K., J.H.-G., A.B., P.J.G., M.H., R.U., L.W., H.E., and M.K. designed the research. M.A. and P.F. performed the research. All authors analyzed the data.

Data Availability Statement. Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data sharing policy described at: https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure

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