Received: 4 September 2020 | Revised: 10 March 2021 | Accepted: 13 March 2021

DOI: 10.1111/bcp.14933

ORIGINAL ARTICLE

Concentration-QT modelling of the novel DHFR inhibitor P218 in healthy male volunteers

Jörg Täubel[1,2] | Ulrike Lorch[1] | Georg Ferber[3] | Christopher S. Spencer[4] | Anne Freier[4] | Simon Coates[1] | Myriam El Gaaloul[5] | Cristina Donini[5] | Mohamed Farouk Chughlay[5] | Stephan Chalon[5]

[1]Richmond Pharmacology Ltd, London, UK

[2]Cardiovascular and Cell Sciences Research Institute, St George's University of London, London, UK

[3]Statistik Georg Ferber GmbH, Riehen, Switzerland

[4]Richmond Research Institute, St George's University of London, London, UK

[5]Medicines for Malaria Venture, Geneva, Switzerland

Correspondence
Jörg Täubel, Richmond Pharmacology Ltd, St George's University of London, Cranmer Terrace, London SW17 0RE, United Kingdom.
Email: j.taubel@richmondpharmacology.com

Stephan Chalon, Medicines for Malaria Venture, ICC Building, Route de Pré-Bois 20, Post Box 1826, CH-1215 Geneva 15, Switzerland.
Email: chalons@mmv.org

Funding information
Janssen PharmaceuticalsMedicines for Malaria Venture

Aims: Given the increasing emergence of drug resistance in *Plasmodium*, new antimalarials are urgently required. P218 is an aminopyridine that inhibits dihydrofolate reductase being developed as a malaria chemoprotective drug. Assessing the effect of new compounds on cardiac intervals is key during early drug development to determine their cardiac safety.

Methods: This double-blind, randomized, placebo-controlled, parallel group study evaluated the effect of P218 on electrocardiographic parameters following oral administration of seven single-ascending doses up to 1000 mg in 56 healthy volunteers. Participants were randomized to treatment or placebo at a 3:1 ratio. P218 was administered in the fasted state with standardized lunch served 4 hours after dosing. 12-lead ECGs were recorded in triplicate at regular intervals on the test day, and at 48, 72, 120, 168, 192 and 240 hours thereafter. Blood samples for pharmacokinetic evaluations were collected at similar time points. Concentration-effect modelling was used to assess the effect of P218 and its metabolites on cardiac intervals.

Results: Concentration–effect analysis showed that P218 does not prolong the QTcF, J-Tpeak or TpTe interval at all doses tested. No significant changes in QRS or PR intervals were observed. Two-sided 90% confidence intervals of subinterval effects of P218 and its metabolites were consistently below the regulatory concern threshold for all doses. Study sensitivity was confirmed by significant shortening of QTcF after a meal.

Conclusion: Oral administration of P218 up to 1000 mg does not prolong QTcF and does not significantly change QRS or PR intervals, suggesting low risk for drug-induced proarrhythmia.

KEYWORDS
antimalarial, aminopyridine, cardiac safety, malaria, P218

Principal investigator: The authors confirm that the Principal Investigator for this paper is Dr Ulrike Lorch and that she had direct clinical responsibility for patients.

1 | INTRODUCTION

Malaria is a life-threatening infection caused by the *Plasmodium* parasite. In 2018, an estimated 219 million cases of malaria were reported, but little progress to reduce global disease incidence has been made since,[1] in part due to the continued emergence of parasite drug resistance which has impacted global efforts to control the disease.[2] More recently, resistance to **artemisinins** was reported[3,4] undermining the chemoprotective potential of artemisinin combination therapy. To meet medical demand, the development of new chemoprotective antimalarials is urgently required.

P218 (3-(2-{3-[(2,4-diamino-6-ethylpyrimidin-5-yl)oxy]propoxy}phenyl)propanoic acid) is an aminopyridine with three downstream metabolites: P218 β acyl glucuronide conjugate, P218 OH, and P218 OH β acyl glucuronide conjugate. P218 is chemically related to the marketed drug **pyrimethamine** (Figure 1). Pyrimethamine is used in combination with sulfadoxine, for the prevention of malaria in children and pregnant women in Africa. Current malaria chemoprotection strategies are therefore dependent on the efficacy of this combination. However, it is no longer being used in many affected areas following the emergence of resistant *Plasmodium* strains. P218 exhibits the same mechanism of action as pyrimethamine; specifically, it selectively inhibits the *Plasmodium* **dihydrofolate reductase** (DHFR) enzyme. The enzyme reduces folates to tetrahydrofolates which are essential for *Plasmodium* DNA biosynthesis.[5] As such, DHFR has emerged as an attractive target for antimalarials. Administration of P218, when used in combination with sulfoxadine, is less likely to lead to the development of resistance, compared with the administration of a single drug.

Quinoline and structurally similar antimalarial drugs have long been associated with cardiovascular side effects.[6] **Halofantrine** is a quinoline-related drug which was considered well tolerated for use for several years. However, the discovery of halofantrine's cardiotoxicity and subsequent reports of its potentially life-threatening adverse cardiovascular effects led to the drug being abandoned as a treatment for malaria.[7–9] Though pyrimethamine and pyrimethamine-related compounds have not been observed to induce cardiotoxic effects,[6] the case of halofantrine emphasizes the need for all antimalarial drugs to be thoroughly investigated for their cardiac impact.

Drug side effects on cardiac repolarization are often mediated through **human Ether-à-go-go** (hERG) channel proteins. In in vitro patch clamp experiments on HEK293 cells, expressing hERG channels tested concentrations of P218 up to 100 μM, which is far in excess (EC_{50} = 56 ± 20 nM for resistant *P. falciparum*[5]) of the recommended therapeutic dose in humans. These concentrations did not adversely affect hERG tail currents (data available on request). According to first-in-human clinical testing, the safety and tolerability of oral P218 are favourable.[10] Furthermore, preclinical assessments of P218 in dogs[11] to determine qualitative electrocardiogram (ECG) variations from baseline after dosing highlighted a lack of cardiovascular risk; administration of P218 had no effect on cardiac conduction times, arterial blood pressure or heart rate in dogs at single doses of up to 50 mg/kg (data available upon request).

What is already known about this subject

- Drug resistance has emerged in malaria parasites against multiple drug classes.
- P218 is a novel pyrimethamine-related drug which targets *Plasmodium* dihydrolate reductase in malaria parasites.
- Cardiac testing is required to confirm the safety of new malaria drugs.

What this study adds

- Oral administration of P218 does not have a significant effect on QTcF, PR, QRS, J-Tpeak, or TpTe interval at single-ascending doses of 10, 30, 100, 250, 500, 750 and 1000 mg.
- Oral administration of up to 1000 mg P218 does not have an effect on QRS or PR intervals.

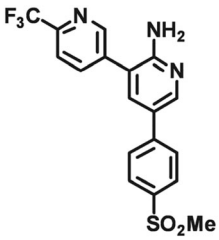

FIGURE 1 Structural formula of P218

In this paper, we report the results of a concentration–effect model-based analysis investigating the effect of P218 on the QTc and its subintervals, QRS and PR intervals in healthy volunteers from a completed first-in-human study.[10] The primary objective of this analysis was to document the effect of this new DHFR inhibitor on cardiac repolarization[12] to further assess the risk/benefit profile of this novel compound for malaria chemoprotection strategies in endemic areas where extended coverage of 3–4 months, matching the malaria season, is typically required.

2 | METHODS

2.1 | Objectives

The objective of this analysis was to assess the effects of single-ascending oral doses of P218 on the QTc, QRS and PR intervals, and electrocardiogram (ECG) morphology in healthy adult volunteers. Concentration–effect modelling was used to determine dose-dependent effects of P218 and its metabolites (observed preclinically,

and confirmed to be major metabolites in humans according to MIST guidance[10]) on QTc and its subintervals.

2.2 | Study design

The first-in-human study used for this analysis was a double-blind, randomized, placebo-controlled, parallel group, ascending-dose study in healthy volunteers (NCT02885506) conducted from August 2016 to December 2017, at Richmond Pharmacology, St George's University of London, London, UK. The study was approved by the local NHS Ethics Committee (South Central—Berkshire B, UK) and the Medicines and Healthcare products Regulatory Authority (MHRA) (London, UK). It was conducted in accordance with Good Clinical Practice and the ethical principles of the Declaration of Helsinki. Written and signed informed consent was obtained from each volunteer before they took part in the study.

Seven incremental dose levels of P218 were evaluated in seven cohorts to assess the drug's safety, tolerability and pharmacokinetics (PKs). The study's ability to detect a small QTc effect, i.e. demonstrating assay sensitivity, was tested by the effect of a meal on the corrected QT interval (QTc).[13]

2.3 | Study participants

Healthy males and females of non-childbearing potential aged 18–45 years with a body weight of at least 50 kg and corresponding body mass index of 18–25 kg/m^2 were eligible to enrol in this study. Screening procedures were conducted within 28 days of study initiation.

Medical histories were taken at enrolment. Volunteers were excluded if they had any clinically important abnormalities in rhythm, conduction or morphology of resting ECG that might have interfered with the interpretation of QTc interval changes. These included sinus node dysfunction, clinically significant PR (PQ) interval prolongation, intermittent second- or third-degree atrioventricular block, incomplete or complete bundle branch block, abnormal T-wave morphology, and prolonged Bazett's-corrected QT[14] (QTcB) > 450 ms or shortened QTcB < 350 ms. Additionally, volunteers were excluded if they had a history or presence of cardiac syncope, recurrent idiopathic syncope, exercise-related clinically significant cardiac events, or a family history of long-QT syndrome.

2.4 | Randomization and masking

Volunteers were randomized to either P218 or a placebo in a 3:1 ratio. Treatment identity was concealed by identical packaging and appearance, odour and taste of both P218 and placebo. The randomization schedule was generated by a statistician using SAS PROC Plan.

2.5 | Procedures

Within each of the seven dose cohorts, six volunteers received a single dose of P218, and two volunteers received a single dose of matching placebo. Volunteers were admitted to the study unit on Day −1 and were randomly assigned a treatment regimen on Day 1. A single dose of 10, 30, 100, 250, 500, 750 and 1000 mg of P218 was administered on Day 1 under fasted conditions. Volunteers were discharged on Day 4 and attended the unit for an outpatient visit on Days 6, 8, 9, and a follow-up visit 11 days post-dose (Figure 2). Treatment was fully supervised by clinical staff.

The reference meal for the assessment of assay sensitivity on Day 1 was lunch, which was served 4 hours after dosing. It contained 1021.3 kcal in a ratio of 40.8% carbohydrate to 36.7% fat to 22.4% protein. Dinner was provided approximately 9–10 hours after dosing and an evening snack was permitted after that.

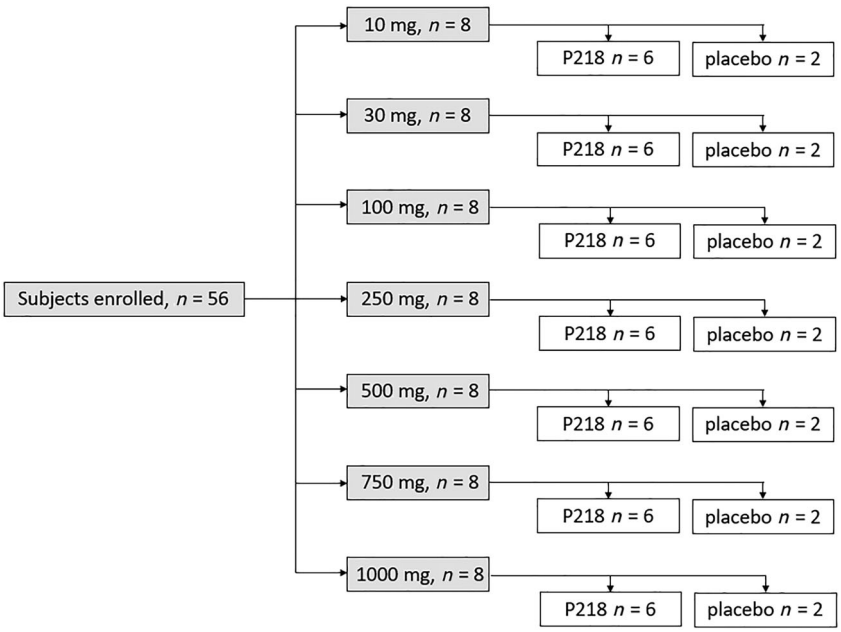

FIGURE 2 CONSORT diagram

2.6 | ECG assessments and QTc evaluation

ECG assessments using 12-lead ECGs were recorded using a GE Marquette MAC1200/MAC1200ST (GE Healthcare, US) and stored electronically on the MUSE information system (GE Healthcare, US). All leads used were superimposed. For subintervals, Lead II was used. ECG recordings were collected at −2, −1, −0.5, 0.5, 1, 2, 4, 6, 7, 8, 12, 24, 48, 72, 120, 168, 192 and 240 hours. All ECG recordings were performed after the volunteers had rested in a supine position for at least 10 minutes. At each time point, ECGs were recorded in triplicate at 1-minute intervals to reduce variance and improve precision of the measurement. Individual ECG recordings lasted 10 seconds. Only ECGs recorded electronically at a stable heart rate (HR) were valid for QT-interval measurements.

Each electronic ECG data file contained the ECG data and the result of the automated ECG analysis performed by the Marquette 12SL ECG Analysis Program. All ECGs and their associated automated interval measurements were subsequently reviewed by qualified cardiologists in accordance with ICH guidance[15] before being used in the statistical ECG analysis. Uncorrected QT interval, RR interval, HR, PR interval, presence or absence of U-waves, quantitative and qualitative ECG variations were assessed by a consultant cardiologist with extensive experience in manual QT measurement, and only QTcF was adjudicated All ECGs were over-read by the same cardiologist. Compensation for HR was applied in order to correct the QT interval (Fridericia correction) (QTcF)[16] and the JT interval (J-Tpeakc).[17] Predose baseline values were obtained from three pre-dose time points (−2, −1, and −0.5 hours before drug administration) in all volunteers, who had fasted for at least 8 hours. In order to reduce variability and in agreement with the recommendations in Garnett et al., the mean of the values obtained at these time points was used as the baseline.[18]

2.7 | Pharmacokinetic assessments

Blood samples for plasma PK evaluation were collected at −0.5, −0.25, 0.5, 1, 2, 4, 6, 7, 8, 12, 24, 48, 72, 120, 168, 192 and 240 hours relative to dosing. Plasma P218 and metabolite concentrations were analysed by Swiss BioQuant (Reinach, Switzerland) using a validated liquid chromatography–tandem mass spectrometry method. This was developed and satisfactorily validated for the measurement of all analytes in human plasma over the calibration range 0.200–200 ng/mL; samples with values greater than >200 ng/mL were diluted with blank human plasma and corrected for the dilution factor. The assay was validated as the relative standard deviation (percentage coefficient of variation [% CV]), the intra- and inter-batch precision did not exceed 15% (range 4.2–6.9%) and the accuracy of the mean determined concentrations to the nominal concentrations of analytes did not exceed 15% (range 98.0–100.7%). Samples, blanks, calibration and quality control solutions were stored at −85 ± 5°C and thawed unassisted in an ice bath. All solutions were satisfactorily stable at bench-top, during freeze–thaw and in −85°C storage for the time frame of the analysis, as determined during the validation of the liquid chromatography–tandem mass spectrometry method. Plasma concentrations of P218 HCl and its metabolites—P218 beta glucuronide hydrochloride, P218-OH and HO-P218 beta glucuronide - hydrochloride—were determined using P218-d4 hydrochloride, P218-d4 beta glucuronide hydrochloride, P218-OH-d4 and P218-HO-d4 beta glucuronide hydrochloride as internal standards. The quantification of the analytes was performed by Hypersil Gold, 2.1 × 50 mm, 3 µm (Thermo Fisher Scientific Inc., Waltham, MA, USA) column separation with reverse-phase chromatography followed by

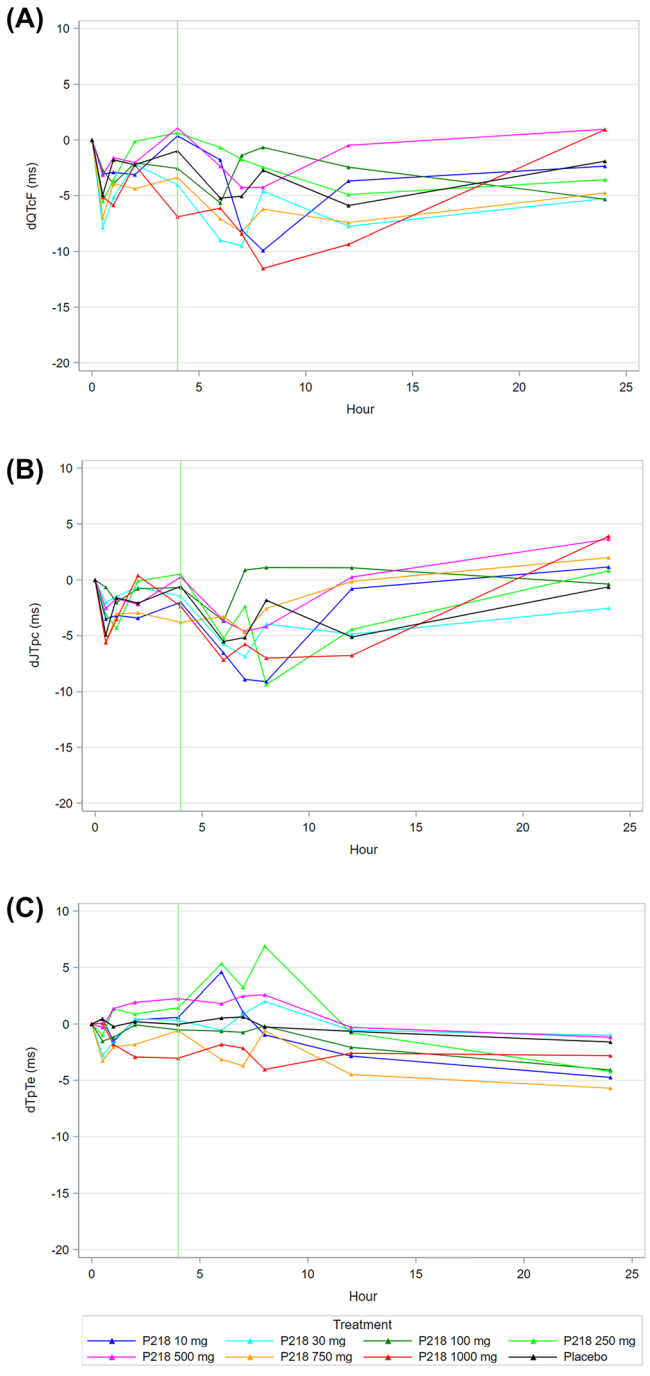

FIGURE 3 Mean change from baseline (A) QTcF; (B) J-Tpeakc and (C) TpTe by treatment group and time point. The plots show no significant changes in cardiac subintervals upon administration of P218

detection with triple-stage quadrupole tandem mass spectrometry in the selected reaction monitoring mode.

2.8 | Statistical analysis

The analysis was based on subinterval and concentration assessments up to 24 hours after volunteers had received P218. All volunteers receiving the study medication who met the following criteria were included in the subinterval analysis set: (1) determined concentrations of P218 and its metabolites below the level of quantification, (2) valid subinterval durations able to be obtained, and (3) time between ECG and blood-draw within acceptable bounds (defined as the sum of the allowed deviation of ECG and PK assessments from the nominal time as specified in the study operations manual).

To investigate the concentration–effect on QTcF, corrected J-Tpeak (J-Tpeakc) and Tpeak-Tend (TpTe) intervals, linear models containing parameters corresponding to each analyte were generated. Following Garnett et al.,[19] a discrete time effect with values for each time point and a treatment intercept allowing a difference in intercept between placebo and active drug were included in the model. The time effect allows accommodation of diurnal variations that are not drug related, in particular those related to the ingestion of food. Introducing a treatment-specific intercept (often called "treatment effect") is not biologically plausible since it would indicate a difference

TABLE 1 Slope, time and treatment intercept effect estimates of primary models

Subinterval	Model	Parameter	Estimate	SE	90% CI	
dQTcF	P218-OH only	P218_OH slope [ms per μg/mL][a]	−8.94	9.06	−23.87	5.98
		Treatment intercept [ms]	−0.45	1.44	−2.86	1.95
		DAY1 H0.5 [ms]	−4.50	1.42	−6.85	−2.15
		DAY1 H1 [ms]	−2.63	1.42	−4.99	−0.27
		DAY1 H2 [ms]	−1.44	1.44	−3.83	0.96
		DAY1 H4 [ms]	−1.10	1.42	−3.46	1.26
		DAY1 H6 [ms]	−4.28	1.42	−6.63	−1.92
		DAY1 H7 [ms]	−5.25	1.41	−7.59	−2.90
		DAY1 H8 [ms]	−4.48	1.41	−6.83	−2.13
		DAY1 H12 [ms]	−4.96	1.42	−7.32	−2.60
		DAY2 H24 [ms]	−2.21	1.42	−4.57	0.16
dJ-Tpeakc	P218-OH only	P218_OH slope [ms per μg/mL][a]	−8.35	9.51	−24.49	7.79
		Treatment intercept [ms]	0.60	1.52	−1.96	3.15
		DAY1 H0.5 [ms]	−4.07	1.47	−6.53	−1.62
		DAY1 H1 [ms]	−2.56	1.48	−5.02	−0.10
		DAY1 H2 [ms]	−1.56	1.50	−4.06	0.94
		DAY1 H4 [ms]	−1.26	1.48	−3.72	1.21
		DAY1 H6 [ms]	−5.46	1.47	−7.92	−3.01
		DAY1 H7 [ms]	−5.09	1.47	−7.53	−2.64
		DAY1 H8 [ms]	−4.57	1.47	−7.02	−2.12
		DAY1 H12 [ms]	−3.37	1.48	−5.83	−0.91
		DAY2 H24 [ms]	0.33	1.48	−2.13	2.80
dTpTe	P218-OH-Glucuronide only	P218_OH-glucuronide slope [ms per ng/mL]	−1.72	1.83	−4.73	1.29
		Treatment intercept [ms]	−0.40	1.06	−2.18	1.37
		DAY1 H0.5 [ms]	−0.42	1.03	−2.13	1.29
		DAY1 H1 [ms]	−0.11	1.04	−1.84	1.61
		DAY1 H2 [ms]	0.53	1.05	−1.22	2.27
		DAY1 H4 [ms]	0.51	1.03	−1.20	2.23
		DAY1 H6 [ms]	1.12	1.03	−0.59	2.84
		DAY1 H7 [ms]	0.61	1.03	−1.10	2.32
		DAY1 H8 [ms]	0.87	1.03	−0.85	2.58
		DAY1 H12 [ms]	−1.31	1.03	−3.03	0.40
		DAY2 H24 [ms]	−2.64	1.03	−4.36	−0.92

[a]In order to make absolute estimates values larger, i.e. about 1–100, the concentrations (ng/mL) have been rescaled: P218_OH/1000, i.e. μg/mL; P218/1000000 i.e. mg/mL; SE, standard error; df, degrees of freedom.

between subjects on placebo and those on drug, even if the drug concentration is nought. Therefore, a significant treatment intercept is an indication of model misfit.

For each subinterval, simplified models were obtained by stepwise removal of the concentration covariate with the smallest absolute *t*-value for the slope estimate. The model with the lowest Akaike Information Criterion (AIC)—a quality measure for model selection—was chosen as the primary model for each subinterval. Additionally, models incorporating only parent P218 concentration were added for post-hoc analyses. For QTcF and J-Tpeakc, the primary models were found to be P218-OH only, and for TpTe it was P218-OH-glucuronide only.

The cardiac safety analysis was conducted using the statistical methods described by Garnett et al.[19] and Ferber et al.[20] The QT interval was corrected using Fridericia's formula[16]; the J-Tpeakc interval was corrected by Johanneson's method[17]; the TpTe interval was not corrected. All ECG parameters were described by summary statistics.

2.9 | Absence of hysteresis

A key prerequisite for the applicability of the models described above is the absence of hysteresis. To identify any PK-pharmacodynamic hysteresis, that is, a delay between the effect of a drug on the QTcF and the plasma concentrations of the drug and its analytes, the analyte concentration was plotted against each subinterval for the highest two dose groups (750 mg and 1000 mg). Hysteresis can be assumed if the ΔΔQTcF estimate exceeds 10 ms at at least one time point in at least one of the two highest dose groups, and a delay of mean ΔΔQTcF is seen with respect to all moieties. A delay in the QT effect would be confirmed if counter-clockwise hysteresis were observed in these plots.[19,21]

2.10 | Assay sensitivity

Tests for assay sensitivity were performed on the basis of the estimates of the time course of ΔQTcF obtained from the primary model described above. In healthy individuals, the QTc interval significantly shortens by about 5.6 ms in the hours following a meal.[13] The shortening effect of a meal on QTc interval has previously been shown to be a viable solution to determine assay sensitivity.[13,20,22]

Volunteers were dosed at fasting state and given lunch 4 hours post-dose. To evaluate assay sensitivity, QTcFs at time points 2, 3 and 4 hours after lunch, i.e. time points 6, 7 and 8 hours post-dose, were used. The change from the average of the last two time points before lunch, i.e., 2 and 4 hours after dosing, was calculated and tested against zero at the one-sided 5% level. To adjust for multiplicity, the Hochberg correction[23] was applied.

2.11 | 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.[24]

3 | RESULTS

3.1 | Volunteer disposition and demographics

A total of 56 healthy volunteers were randomized and dosed according to the clinical study protocol. Despite being open to both male and female volunteers, the final pool of enrolled participants was

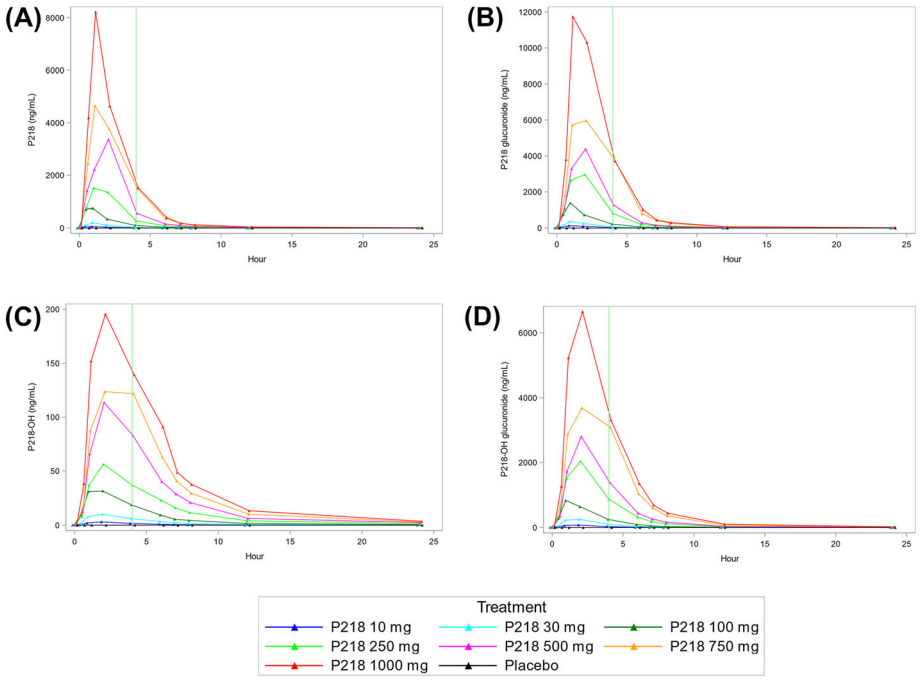

FIGURE 4 Plasma concentration time course for (A) P218; (B) P218 glucuronide; (C) P218-OH and (D) P218-OH glucuronide

male only. All 56 volunteers completed the study as per the protocol. Demographic data and volunteer disposition are summarized in Supplementary Table S1.

3.2 | Cardiac assessments

All volunteers in the safety data set with valid ECG data were included in the primary analysis. One time point was excluded due to an implausible ECG time. Because all values were taken in triplicate, three ECGs were excluded from a total of 2016 recordings. Therefore, the final number of ECGs used for modelling was 2013.

The QTcF, J-Tpeakc and TpTe values for the placebo and the seven dose cohorts were similar for the test duration of 24 hours (Figure 3A–3C). Maximal mean QTcF values for P218 doses of 10 mg, 30 mg, 250 mg, 500 mg and placebo were observed between 1 and 4 hours. Maximal mean QTcF values for a dose of 100 mg were detected at 8 hours, and at 24 hours for 1000 mg post-dose. Minimum mean values were observed between 6 and 12 hours post-dose.

Maximal mean J-Tpeakc values for all doses and placebo were observed between 8 hours and 24 hours post-dose, except for 30 mg, for which the maximal value was reached at 2 hours post-dose. Minimal mean values were observed between 6 and 12 hours post-dose.

For TpTe, the maximal mean values for all doses and placebo were detected between 6 and 8 hours post-dose, except for 100 mg, for which the maximal value was obtained at 2 hours post-dose, and for 1000 mg the maximum was reached at 0.5 hours post-dose. Minimal mean values were observed at 24 hours post-dose, except for the 30 mg dose for which the minimal mean value was detected at 1 hour.

RR intervals exhibited an increase at around 6–12 hours post-dose, correlating with the effect of food on HR,[22] and were not due to dosing with P218. Time courses of HR and QTcF showed no systemic differences between groups receiving P218 and those receiving a placebo (Supplementary Figure S1). Therefore, no-drug-related changes were observed.

3.3 | Model-based concentration–effect analyses

Modelling here was performed using data collected from all dose concentrations, but predictions were made on the concentrations seen in the two highest doses. Details of the model selection procedure for QTcF can be seen in Supplementary Table S2, which shows calculated AIC values for all tested models. For QTcF, the primary model (i.e., those with the lowest AIC score) were found to be P218-OH only. A similar procedure for JTpeakc and TpTe resulted in a model based on P218-OH for JTpeakc while for TpTe it was P218-OH-glucuronide only (Table 1). Plasma concentration time course plots for P218 and its major metabolites are shown in Figure 4. Geometric mean C_{max} values at the highest dose were 8642 ng/mL, 193 ng/mL and 6570 ng/mL for P218, P218-OH and P218-OH glucoronide, respectively. There was no indication of a delayed effect on QTcF; thus, hysteresis could be excluded (Supplement Figure S2).

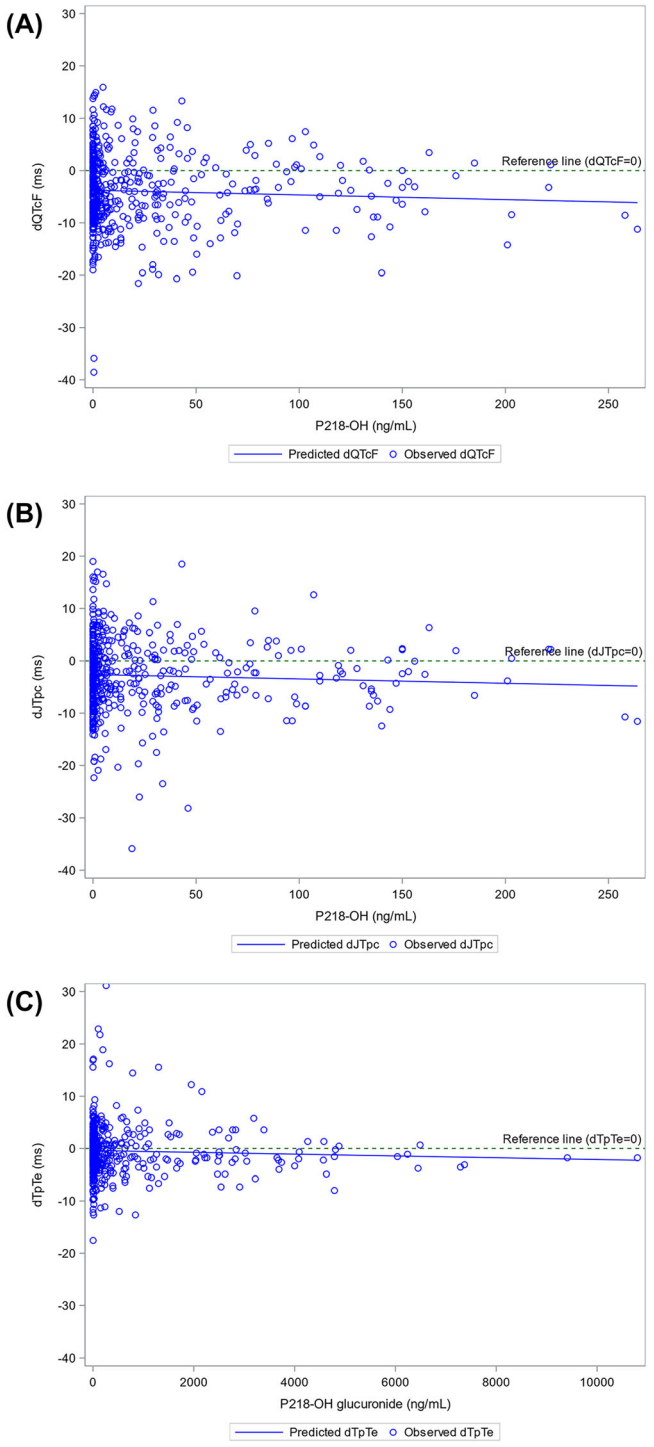

FIGURE 5 Scatterplots for primary and parent models and model molar sums. (A) ΔQtCF primary model; (B) ΔJ-Tpeakc primary model; (C) ΔTpTe primary model. The plots suggest a lack of dose-dependent response of P218 on cardiac subintervals

3.3.1 | QTcF

In the primary model, a slightly negative, but non-significant relationship between the concentration of P218-OH and change in QTcF was seen. The scatterplot in Figure 5A displays the ΔQTcF values over the concentration of this metabolite.

TABLE 2 QTcF prolongation—Effect of a meal

Model	Status	Hours after start of meal	Effect estimate	SE	95% CI	
P218_OH only	Primary	2	−2.97	1.20	−5.34	−0.60
		3	−3.92	1.26	−6.41	−1.43
		4	−3.15	1.30	−5.71	−0.60
Parent only	Ad hoc	2	−2.81	1.27	−5.31	−0.31
		3	−3.70	1.28	−6.22	−1.17
		4	−2.90	1.29	−5.44	−0.36

SE, standard error; df, degrees of freedom.

3.3.2 | J-Tpeakc

In the primary model, a slightly negative, but non-significant relationship between the concentration of P218-OH and the change in J-Tpeakc was detected (Figure 5B).

3.3.3 | TpTe

A slightly negative, but non-significant relationship was found between the concentration of P218-OH-glucuronide and ΔTpTe (Figure 5C).

Treatment effect sizes confirmed that any QTc changes were driven by the moiety dose rather than the time of day. Collectively, the modelling showed no significant dose-dependent effect on QTc or its subintervals. The slopes of the models were negative throughout, −8.94 ms per μg/mL for QTcF, −8.35 ms per μg/mL for JTpeakc and −1.72 ms per mg/mL for TpTe.

3.4 | Sensitivity of the assay

The sensitivity of the assay to consistently detect changes in subinterval duration was established by measuring the effect of ingesting a standardized meal. As can be seen in Figure 3, QTcF consistently dropped at time points 2, 3 and 4 hours after lunch, i.e. 6, 7 and 8 hours after drug administration. This trend was similar in the placebo and treatment groups. Table 2 shows that shortening at the timepoints considered was consistently significant, i.e. the two-sided 95% confidence interval excluded 0, and therefore provides validation that the experiment is able to detect small changes in QTc.

4 | CONCLUSION

This analysis, based on a first-in-human study by Chughlay et al.,[10] found that P218 and its major metabolites did not prolong QTcF, J-Tpeakc or TpTe following administration of single doses up to 1000 mg across seven single-ascending doses in healthy men. Further, no significant changes in QRS or PR intervals were observed. Our findings highlight that exposure to P218 or its metabolites had no effects on cardiac repolarization at the plasma levels tested. At the highest dose of 1000 mg, the mean plasma concentration was 8642 ng/mL and the largest value observed in any individual was 14 800 ng/mL.[10] Both the mean and the largest value were far in excess of the known EC_{50} for P218. The EC_{50} value was estimated using a parasite growth assay to establish the concentration required to inhibit 50% of parasite growth *in vitro* and was calculated to be 4.6 ± 1.9 nM for susceptible *P. falciparum* and 56 ± 20 nM for resistant *P. falciparum*.[5] Additionally, the rate of clearance for P218 was 56.13 L/h for doses of 1000 mg in healthy subjects.[10] Metabolism is not hepatic stage 1 metabolism, rather it is largely glucuronidation. It is possible that glucuronidation enzyme inhibiting drugs may influence phase 2 metabolism of the drug. P218 is eliminated via urinary and biliary excretion. At this stage, it is difficult to make a quantitative assessment of the impact on C_{max} if clearance is impaired. Taken together, these data indicate that an efficacious dose would likely be devoid of adverse cardiac events. Moreover, PK modelling predicts that two 100 mg oral P218 doses administered 48 hours apart would determine the lowest efficacious P218 exposure associated with malaria chemoprotection in humans. Further proof of clinical pharmacology studies are ongoing (registered with the *clinicaltrials.gov* identifier: NCT03707041) and aimed at confirming these PK modelling predictions and to establish the upper bound for efficacy strategies in field trials. The primary models indicated a non-significant negative slope. The reverse was true of the parent model, indicating that the treatment effect was negligible. The lack of an effect of P218 on cardiac conduction/repolarization may be indicative of other antimalarial drugs of the aminopyridine class.

P218 is rapidly metabolized into P218 beta-acyl glucuronide, P218-OH and P218-OH beta-acyl glucuronide. The two glucuronide species were considered equally chemically reactive given their acyl glucuronide structure, resulting in a similar effect on cardiac repolarization. Modelling determined that none of these chemically reactive species have arrhythmogenic potential. All HR changes were related to food administration in line with previously observed data,[13,20,22] no significant chronotropic effect of P218 was detected in this study.

Rather than using moxifloxacin as a positive control to observe QT interval prolongation, this study employed the food effect to demonstrate assay sensitivity. The robust shortening in QT interval of

around 5 ms observed after eating a meal is an established positive control[20,25] to ensure that the assay is sensitive enough to show a drug effect at the 5 ms threshold of regulatory concern.[26,27] The observed food effect successfully validated the sensitivity of the study to detect a small effect on QTc of around 5 ms. While the small size of each dose group can distort estimates of the drug effect by time point, concentration–QTc modelling uses information across time points and dose groups in a single model and prediction, and therefore offers more precise estimates (provided the model assumptions are met).

Though the study design targeted healthy males and females, our final cohorts consisted of only male participants. Given the known differences in cardiac electrophysiology between males and females,[28,29] this presents a potential limitation. Previously observed differences in electrophysiology suggest that females tend to have a longer QT interval, with a more pronounced effect of K^+ channel block on the J-Tpeak subinterval. When QT-prolonging drugs were administered, females were shown to have a slightly exaggerated QT prolongation compared to males.[30] However, it has not previously been observed that a drug-derived QTc change occurred in only one sex and not the other. Thus, it is does not seem likely that there would be a QTc effect in females as a result of P218 administration.

A preclinical efficacy study in *Plasmodium falciparum*-infected, humanized mice predicted an IC_{50} for P218 of 4.6 nM.[5] When administered to humans, P218 was observed to have C_{max} concentrations for all dose levels in the pharmacologically relevant range. It was also demonstrated that P218 was generally tolerated as a single dose up to 1000 mg in healthy volunteers.[10] Its efficacy suggests that the treatment has potential to be used as a novel candidate for malaria chemoprotection. Due to the short $T_{1/2}$ observed in the first-in-human study,[10] work is ongoing to identify a modified-release formulation which can maintain the plasma concentration of the drug for longer, so that a single weekly or monthly dose may achieve chemoprotectivity.

In conclusion, neither P218 nor its metabolites induced clinically relevant changes in cardiac conduction/repolarization up to a single oral dose of 1000 mg. In particular, our concentration–effect modelling shows no drug-induced QTc prolongation with this new antimalarial. Given its positive clinical safety and tolerability profile previously documented, this novel DHFR inhibitor may allow for the delivery of safe and effective chemoprotection against malaria.

ACKNOWLEDGEMENTS

The authors thank Janssen Pharmaceuticals, Belgium, and BIOTEC, Thailand, for their ongoing collaboration with Medicines for Malaria Venture (MMV) in the development of P218. We thank all the volunteers who participated in the study. We thank the Medicines for Malaria Venture for assisting with the experimental conception, writing and feedback. We thank the clinical and support staff at Richmond Pharmacology Ltd for their invaluable assistance in the execution of the clinical study. This study was funded by Medicines for Malaria Venture, Geneva, Switzerland, and Janssen Pharmaceuticals, Beerse, Belgium. Janssen Pharmaceuticalscomments were non-binding. Those individuals at MMV who commented on the manuscript are listed as authors on the manuscript itself.

COMPETING INTERESTS

J.T. and U.L. are employees of Richmond Pharmacology Ltd. G.F. is an employee of Statistik Georg Ferber GmbH. C.S. and A.F. are employees of the Richmond Research Institute. M.E.G., C.D., M.F.C. and S.Ch. are employed by Medicines for Malaria Venture. P218 is being developed by Medicines for Malaria Venture, who provided funding for this publication.

CONTRIBUTORS

J.T., U.L., M.E.G., C.D., M.F.C. and S.Ch. conceived the study. G.F. analysed the data. C.S. and A.F. oversaw the preparation of the manuscript. All authors approved the final submitted version and agreed to the publication.

DATA AVAILABILITY STATEMENT

Requests for access to data should be addressed to the corresponding authors.

ORCID

Jörg Täubel https://orcid.org/0000-0002-0010-207X
Ulrike Lorch https://orcid.org/0000-0002-6316-5860
Christopher S. Spencer https://orcid.org/0000-0002-9552-0281
Mohamed Farouk Chughlay https://orcid.org/0000-0002-7972-1710

REFERENCES

1. World Health Organization. World malaria report 2019. https://www.who.int/publications/i/item/9789241565721. Accessed October 10, 2020.
2. Cui L, Mharakurwa S, Ndiaye D, Rathod PK, Rosenthal PJ. Antimalarial drug resistance: literature review and activities and findings of the ICEMR network. *Am J Trop Med Hyg*. 2015;93(3_Suppl):57-68.
3. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Evidence of artemisinin-resistant malaria in western Cambodia. *N Engl J Med*. 2008;359(24):2619-2620.
4. Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med*. 2009;361(5):455-467.
5. Yuthavong Y, Tarnchompoo B, Vilaivan T, et al. Malarial dihydrofolate reductase as a paradigm for drug development against a resistance-compromised target. *Proc Natl Acad Sci USA*. 2012;109(42):16823-16828.
6. White NJ. Cardiotoxicity of antimalarial drugs. *Lancet Infect Dis*. 2007;7(8):549-558.
7. Nosten F, Ter Kuile FO, Luxemburger C, et al. Cardiac effects of antimalarial treatment with halofantrine. *Lancet*. 1993;341(8852):1054-1056.
8. Castot A, Rapoport P, Le Coz P, et al. Prolonged QT interval with halofantrine. *Lancet*. 1993;341(8859):1541-1542.
9. Bouchaud O, Imbert P, Touze JE, Dodoo AN, Danis M, Legros F. Fatal cardiotoxicity related to halofantrine: a review based on a worldwide safety data base. *Malar J*. 2009;8(1):289.
10. Chughlay MF, Rossignol E, Donini C, et al. First-in-human clinical trial to assess safety, tolerability and pharmacokinetics of P218, a novel candidate for malaria chemoprotection. *Br J Clin Pharmacol*. 2020;86(6):1113-1124.

11. Guth BD. Preclinical cardiovascular risk assessment in modern drug development. *Toxicol Sci*. 2007;97(1):4-20.
12. Vicente J, Zusterzeel R, Johannesen L, et al. Assessment of multi-ion channel block in a phase I randomized study design: results of the CiPA phase I ECG biomarker validation study. *Clin Pharmacol Ther*. 2019;105(4):943-953.
13. Taubel J, Wong AH, Naseem A, Ferber G, Camm AJ. Shortening of the QT interval after food can be used to demonstrate assay sensitivity in thorough QT studies. *J Clin Pharmacol*. 2012;52(10):1558-1565.
14. Bazett HC. An analysis of the time relations of electrocardiograms. *Heart*. 1920;7:353-370.
15. ICH E14 Implementation Working Group. ICH E14 Guideline: The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs Questions & Answers (R3) 2015 [2/6/2016]. 61134].
16. Fridericia LS. Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken. *Acta Med Scand*. 1921;54(1):17-50.
17. Johannesen L, Vicente J, Gray RA, et al. Improving the assessment of heart toxicity for all new drugs through translational regulatory science. *Clin Pharmacol Ther*. 2014;95(5):501-508.
18. Garnett C, Bonate PL, Dang Q, et al. Scientific white paper on concentration-QTc modeling. *J Pharmacokinet Pharmacodyn*. 2018;45(3):383-397.
19. Garnett CE, Zhu H, Malik M, et al. Methodologies to characterize the QT/corrected QT interval in the presence of drug-induced heart rate changes or other autonomic effects. *Am Heart J*. 2012;163(6):912-930.
20. Ferber G, Wang D, Täubel J. Concentration–effect modeling based on change from baseline to assess the prolonging effect of drugs on QTc together with an estimate of the circadian time course. *J Clin Pharm Ther*. 2014;54(12):1400-1406.
21. Holford NH, Sheiner LB. Understanding the dose–effect relationship. *Clin Pharmacokinet*. 1981;6(6):429-453.
22. Taubel J, Ferber G. The reproducibility of QTc changes after meal intake. *J Electrocardiol*. 2015;48(2):274-275.
23. Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. *Biometrika*. 1988;75(4):800-802.
24. Alexander SPH, Fabbro D, Kelly E, et al. The Concise Guide to PHARMACOLOGY 2019/20: Enzymes. *Br J Pharmacol*. 2019;176(S1):S297-S396.
25. Cirincione B, Sager PT, Mager DE. Influence of meals and glycemic changes on QT interval dynamics. *J Clin Pharmacol*. 2017;57(8):966-976.
26. Sager PT, Nebout T, Darpo B. ICH E14: a new regulatory guidance on the clinical evaluation of QT/QTc internal prolongation and proarrhythmic potential for non-antiarrhythmic drugs. *Drug Inf J*. 2005;39(4):387-394.
27. Darpo B, Nebout T, Sager PT. Clinical evaluation of QT/QTc prolongation and proarrhythmic potential for nonantiarrhythmic drugs: the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use E14 guideline. *J Clin Pharmacol*. 2006;46(5):498-507.
28. Haukilahti MA, Holmström L, Vähätalo J, et al. Sudden cardiac death in women: causes of death, autopsy findings, and electrocardiographic risk markers. *Circulation*. 2019;139(8):1012-1021.
29. Jonsson MK, Vos MA, Duker G, Demolombe S, Van Veen TA. Gender disparity in cardiac electrophysiology: implications for cardiac safety pharmacology. *Pharmacol Therapeut*. 2010;127(1):9-18.
30. Ebert SN, Liu XK, Woosley RL. Female gender as a risk factor for drug-induced cardiac arrhythmias: evaluation of clinical and experimental evidence. *J Womens Health*. 1998;7(5):547-557.

SUPPORTING INFORMATION

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

How to cite this article: Täubel J, Lorch U, Ferber G, et al. Concentration-QT modelling of the novel DHFR inhibitor P218 in healthy male volunteers. *Br J Clin Pharmacol*. 2022;88(1):128–137. https://doi.org/10.1111/bcp.14933