Article Type: Clinical Trial

Title

Pharmacokinetics-Pharmacodynamics of High-Dose Ivermectin with Dihydroartemisinin-Piperaquine on Mosquitocidal Activity and QT-prolongation (IVERMAL).

Authors

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Keywords

Clinical trial, pharmacokinetics, pharmacodynamics, ivermectin, dihydroartemisinin-piperaquine, Malaria, *Plasmodium falciparum*, *Anopheles gambiae s.s.*, QT-interval, Kenya.

Conflicts of interest

The authors declared no competing interests for this work.

Funding

This study is funded by the Malaria Eradication Scientific Alliance (MESA), through a sub-grant from the Bill and Melinda Gates Foundation (BMGF). Neither MESA nor the BMGF had any role in the design of the study, the collection, analysis, and interpretation of data, or in the writing the manuscript. Co-funding was provided by the U.S. Centers for Disease Control and Prevention, through a Cooperative Agreement between CDC and LSTM.

Abstract

High-dose ivermectin, co-administered for 3-days with dihydroartemisinin-piperaquine (DP), killed mosquitoes feeding on individuals for at least 28-days post-treatment in a recent trial (IVERMAL), while 7-days was predicted pre-trial. The current study assessed the relationship between ivermectin blood concentrations and the observed mosquitocidal effects against *Anopheles gambiae*. 3-days ivermectin 0, 300, or 600 mcg/kg/day plus DP was randomly assigned to 141 adults with uncomplicated malaria in Kenya. During 28-days follow-up, 1,393 venous and 335 paired capillary plasma samples, 850 mosquito-cluster mortality rates, and 524 QTcF-intervals were collected. Using pharmacokinetic-pharmacodynamic (PK-PD) modeling, we show a consistent correlation between predicted ivermectin concentrations and observed mosquitocidal-effects throughout the 28-day study duration, without invoking an unidentified mosquitocidal metabolite or drug-drug-interaction. Ivermectin had no effect on piperaquine's pharmacokinetics or QTcF-prolongation. The PK-PD model can be used to design new treatment regimens with predicted mosquitocidal effect. This methodology could be used to evaluate effectiveness of other endectocides.

Introduction

Mass drug administration (MDA) with the long-acting antimalarial dihydroartemisinin-piperaquine (DP) is being evaluated in several malaria endemic countries for malaria transmission reduction and elimination.[1-3] Ivermectin is an antiparasitic drug, which also kills mosquitoes feeding on recently treated individuals. Adding ivermectin to DP has been proposed as an innovative tool to increase the impact of MDA for malaria.[4] However, the single dose of 150-200 mcg/kg ivermectin used for onchocerciasis and lymphatic filariasis control has only a small and short-lived effect (<7 days) on mosquito mortality.[5] Ivermectin is documented to be remarkably well tolerated, even up to doses of 2,000 mcg/kg.[6, 7]

In a recent randomized, double-blind, placebo-controlled trial in western Kenya, ivermectin 300 and 600 mcg/kg/day, co-administered for 3 days with DP, were shown to kill mosquitoes feeding on individuals for at least 28-days post-treatment.[8] This was significantly longer than the 7-day effectiveness expected based on the predicted time ivermectin would remain above the *in vitro* median lethal concentration (LC_{50}) for *Anopheles gambiae* in pre-trial simulations.[5] Two possible explanations are an unidentified mosquitocidal metabolite with a longer active half-life than the parent compound ivermectin or a drug-drug interaction that results in a slower clearance of ivermectin from the circulation. As ivermectin and piperaquine are both metabolized by cytochrome P450 3A4 (CYP3A4), it has been hypothesized that an interaction could occur.[5] Using the trial data, the current pharmacokinetic and pharmacodynamic analysis aimed to determine whether a drug interaction or an unidentified ivermectin metabolite could be contributing to the prolonged mosquitocidal effect of ivermectin.

Results

Ivermectin PK

Ivermectin pharmacokinetics were best described by a two-compartment oral absorption model (***Figure S1***, ***Table S1***). The initial sequential model, utilizing only plasma concentration data to fit the PK data, resulted in the parameters described in ***Table 1***. Goodness-of-fit plots are shown in ***Figure 1*** (observed capillary-venous plots are shown in ***Figure S2)***. In the ivermectin arms post-treatment, concentrations were below the limit of quantification (BLQ) for 277 of 805 (34.4%) venous and 44 of 224 (19.6%) capillary samples, predominantly at later time points (***Table S2***).

The subsequent simultaneous PK-PD model resulted in remarkably similar PK parameters to the initial sequential approach (***Table 1)***, meaning the PD element did not disturb the PK prediction, which supports the notion that it is unnecessary to invoke an active metabolite to explain the pharmacodynamic output of ivermectin.

Piperaquine PK

Piperaquine pharmacokinetics were best described using a three-compartment oral absorption model (***Figure S1***, ***Table S1***), resulting in the parameters displayed in ***Table 1.*** Concentrations were below the limit of quantification (BLQ) for 3 of 1248 (0.2%) venous and 0 of 333 (0%) capillary post-treatment samples (***Table S2***). Goodness-of-fit graphs including both capillary and venous concentrations are shown in ***Figure 2*** (observed capillary-venous plots are shown in ***Figure S2***). A visual predictive check, incorporating variation in patient weights, resulted in a profile which accurately fits the observed population pharmacokinetic data (***Figure 2***, e). Post-hoc analysis showed that piperaquine PK was not influenced by ivermectin with $\boldsymbol{AUC}$, $\boldsymbol{C_{max}}$, terminal $\boldsymbol{t_{1/2}}$ (***Table 1***) and the overall PK profile (***Figure 2***, f) showing no differences across all three study arms.

Ivermectin PD

Pharmacodynamic analysis of ivermectin activity was performed initially by building an exposure-effect relationship between pooled observed venous ivermectin concentrations in patients and the corresponding mosquitocidal activity of each blood sample ***(Figure 3,*** a***).*** This generated ivermectin's predicted half-maximal effective concentration ($\boldsymbol{EC_{50}}$) of 11.3 ng/mL and maximal mosquito mortality rate ($\boldsymbol{E_{max}}$+ $\boldsymbol{E_{min}}$) of 48.8 deaths per 100 mosquito days observed (an incidence density rate). Baseline mortality rate ($\boldsymbol{E_{min}}$) was fixed to 3.7 deaths/100 days based on mortality rates observed in 392 mosquito clusters feeding on ivermectin-free blood from baseline and control samples.

The relationship between mosquitocidal activity and pooled predicted ivermectin concentrations from the sequential PK-PD model (including 321 BLQ venous and capillary concentrations that were predicted but not observed) resulted in a similar predicted pharmacodynamic response, compared to analyzing observed data only (***Table 2*** and ***Figure 3***, b). A sequential population method, incorporating individual PK parameters (assessed previously) and individuals' corresponding PD output, gave similar results: predicted median maximal mosquito mortality rate ($\boldsymbol{E_{max}}$+ $\boldsymbol{E_{min}}$) of 57.3 [p5-p95: 42.1-68.8] deaths per 100 mosquito days, median $\boldsymbol{EC_{50}}$ of 20.5 ng/mL [p5-p95: 6.1-49.7], and median $\boldsymbol{E_{min}}$ of 3.84 [p5-p95: 3.6-4.4] deaths per 100 mosquito days. Finally, the simultaneous PK-PD model resulted in pharmacodynamic parameters that are very similar to those obtained from utilizing the sequential models or observed data only (***Table 2*** and ***Figure 3***, c).

The relationship between ivermectin exposure and its mosquitocidal effect was investigated separately for each day of follow-up (days: 2+4h, 7, 10, 14, 21 and 28). As most samples beyond day 14 resulted in unquantifiable concentrations (BLQ), which would have restricted the ability of PD analysis, predicted concentrations from the sequential PK model (using PK data from all days) were used for PD modeling; this was justified as predicted concentrations matched observed values accurately (***Figure 1***, A & ***Figure 3***, B). ***Figure 4*** shows the relationship between predicted ivermectin exposure and mosquito mortality rate at each study visit. The results indicate that the mosquito mortality rate has the same relationship with predicted ivermectin concentration at all post-treatment feeding days of the study, including days 21 and 28 when most ivermectin exposures were BLQ. There is no effect of time post-treatment on the shape of the sigmoidal relationship between predicted ivermectin concentration and observed mortality rate. Similar to this sequential PK-PD approach, the simultaneous PK-PD model showed no bias over time in residual analysis of PD predictions, meaning ivermectin's concentration-effect relationship (i.e. $\boldsymbol{EC_{50}}$ and $\boldsymbol{E_{max}}$) was consistent through-out the study duration (***Figure S4***).

Mosquito mortality was also analyzed as a proportion (%) to calculate the ivermectin concentration able to kill 50% of mosquitoes by a specific time point (LC_{50}), unadjusted and adjusted for baseline mortality (***Figure S3, Table S3***).

Ivermectin PK-PD simulation

A visual predictive check of the PK-PD simulation did not show any bias in comparison with observed PD data in either arm of the study (***Figure 5***).

Piperaquine PD

Piperaquine's effect upon QTcF was analyzed using a conventional linear model as well as a dynamic E_{max} model. Using the linear model, piperaquine resulted in a mean QTcF-prolongation of 3.71 ms (95% CI: 3.12-4.29) per 100 ng/mL with a baseline intercept of 6.76 ms (95% CI: 4.99-8.53) (***Figure S5***). Using the dynamic E_{max} population model, that was simultaneously fitted with piperaquine PK using Pmetrics™, we show that from a median baseline QTcF-interval of 399.3 ms [p5-p95: 377.5-416.3] the maximal ΔQTcF-interval was estimated at 53.5 ms [31.1-122.9], resulting in a maximal QTcF-interval of 449.8 ms [415.1-520.0]. The median concentration required to achieve an effect half-way between baseline and maximal possible prolongation (EC_{50}) was 181.7 ng/mL [16.0-1200.0]. Parasitemia, present at low levels and only at baseline, was not associated with QTcF at baseline versus day 28, at day 2+4h versus day 0, or at day 2+4h versus day 28.

Piperaquine concentration correlated with QTcF (***Figure S5***), however post-hoc analysis showed only a weak ($\rho<0.3$), albeit significant, correlation between piperaquine C_{max} and D2+4h QTcF (***Table S6***). Piperaquine AUC was not correlated with D2+4h QTcF and ΔQTcF (***Table S6***). Ivermectin dose did not modify piperaquine's QTcF-prolonging effect (***Table S4*** and ***Table S5***).

Discussion

Ivermectin's pharmacodynamic effect on *An. gambiae* mortality was much stronger and persisted much longer post-treatment in the current trial than reported in previous studies. Pharmacokinetic-pharmacodynamic analysis showed that the entire 28 days of mosquitocidal effect post-treatment observed in the main trial[8] could be explained by ivermectin concentrations alone, without invoking unidentified variables such as an active metabolite or a drug-drug interaction. Furthermore, ivermectin exerted no modification of piperaquine's pharmacokinetics or QTcF-prolongation. This ivermectin PK-PD model can be used to design new treatment regimens and predict their mosquitocidal effect. The methodology could also be used to assess new endectocides.

Ivermectin PK

Ivermectin pharmacokinetic properties in this study were similar to those reported in earlier studies, which used venous concentrations from adults receiving single doses ranging between 150 and 2,000 mcg/kg,[7, 9-14] and repeated doses up to 1,000 mcg/kg given three-times in 1 week.[7] Ivermectin clearance has been reported as 19.2 L/h (SD ±14.8),[10] 17.3 L/h (SD ±9.2),[14] and in a study with co-administration of azithromycin and albendazole, as 11.8 L/h (RSE 32.8%) overall, and 12.3 L/h (RSE 42.6%) when allowing for different sub-populations.[11] Clearance in the current study using a simultaneous model was 10.9 L/h for an average 60 kg patient (***Table 1***), which is similar to previous studies and makes a drug-drug-interaction in the current study unlikely.

Ivermectin PD

Ivermectin's mosquitocidal effect has been assessed in several *in vitro* studies with spiked blood and three previous clinical trials, one of which measured plasma concentrations. Outcomes are often expressed as the LC_{50} by a specified time post-feeding (e.g. by 7 days), although assay durations vary widely, as does adjustment for background mortality rate, hampering comparability. *In vivo* adjusted LC_{50} values in the current trial were 0.6, 2.6, 4.3, 4.7, and 7.1 times lower than previous studies

(***Figure S3***, ***Table S3***).[15-18] Noticeably there was no major difference between the current study and the previous *in vivo* trial (adjusted LC_{50}: 2.6 vs 6.5 ng/mL), especially considering the later value could be an overestimation due to a scarcity of data in the upper ranges of concentrations (maximum value achieved: 15.6 ng/mL) and mosquito mortality rates (2 of 233 values were >90%).[15] Any major difference between LC_{50} estimates obtained from *in vivo* and *in vitro* studies could suggest the presence of an active metabolite, however, comparisons between the current *in vivo* trial and the *in vitro* studies were inconclusive, some showing either lower (0.6 fold) or higher (7.1 fold) values. Nevertheless, our PK-PD model showed that the entire mosquitocidal effect, including its prolonged effect beyond day 7 up to day 28 post-treatment, could be explained by ivermectin concentrations alone, without the need to invoke an unidentified metabolite (***Figure 5***, ***Table 2, Figure S4***). This indicates that ivermectin in its parent form is likely the sole contributor to the observed mosquitocidal effect for the duration of the study, or if an active metabolite is present, then it must have a remarkably similar pharmacokinetic profile to ivermectin itself.

Ivermectin effect-duration

The 28-day exposure-effect relationship exceeded the prediction from the pre-trial pharmacodynamic simulation which estimated an effect-duration of only 7 days.[5] The simulation was based on previously reported 7-day-LC_{50} value of ivermectin of 15.9 ng/mL,[17] while in the current trial the 7-day-LC_{50} was 3.4 ng/mL. Importantly, this study also illustrated the value of using thresholds below the LC_{50} to help explain the effect-duration, such as 5% and 1% of maximal activity ($\boldsymbol{EC_5}$ 0.83 ng/mL and $\boldsymbol{EC_1}$ 0.16 ng/mL), which were reached at 20.1 and 28.1 days post-treatment with the 3-day 600 mcg/kg/day regimen (***Table 2***). The latter is consistent with the observed 28-day mosquitocidal effects of the main trial.[8] Future pharmacodynamic simulations should consider using these $\boldsymbol{EC_1}$-$\boldsymbol{EC_5}$ concentrations as thresholds to predict effect-durations.

With this PK-PD model we also show that the high-dose regimens can extend the time that 5% of maximal activity against mosquitoes is achieved from 13 days with a single-dose of 400 mcg/kg to 23 days with the 3-day 600 mcg/kg/day regimen; additionally the overall kill rate using the 3-day 600 or 300 mcg/kg/day regimens is predicted to be several fold faster in the first two weeks of treatment when compared to the 400 mcg/kg single-dose (***Figure 5***).

Assessing endectocides

Other aspects of the methodology followed in this analysis may also be useful for further assessment and comparisons of new endectocides and regimen optimization.[19] We propose to collect the following data for each drug: $\boldsymbol{E_{max}}$, $\boldsymbol{E_{min}}$, $\boldsymbol{EC_{50}}$, Hill coefficient (in this study equaling 1), and PK parameters (e.g. $\boldsymbol{CL}$, $\boldsymbol{V}$, $\boldsymbol{Q_1/F}$, $\boldsymbol{V_{P1}/F}$ and $\boldsymbol{k_a}$) to allow for PK-PD simulation. $\boldsymbol{E}$ is specific to the mosquito species studied. We also propose to express the pharmacodynamic effect of each endectocide on mosquito mortality as an incidence density rate (IDR) by day 14 (i.e. deaths per 100 mosquito days observed, during a 14-day mosquito survival assay). The 14-day follow-up is required to capture the cumulative effect during a prolonged extrinsic cycle. The use of IDR's instead of hazards are recommended, as rates, are easier to interpret and incorporate in PK-PD models. For example, in the PK-PD simulation it was possible to simulate an IDR for any desired drug regimen at any time post-treatment. By dividing the drug IDR ($\boldsymbol{E}$) by the baseline mortality rate ($\boldsymbol{E_{min}}$) it was then possible to calculate the incidence rate ratio ($\boldsymbol{IRR} = \boldsymbol{E} / \boldsymbol{E_{min}}$) at each timepoint (see ***IVERMAL Dose-Response Calculator*** and ***Figure 5d***). Both IRR's and HR's assess the relative differences in incidence rates, can be interpreted in roughly the same way,[20] and equally incorporated into population models. As MDA rounds are likely to be spaced at least 1 month apart, determining an endectocide's IRR at 28 days post-treatment (either directly or by simulation) may be a useful measure to assess prolonged effectiveness.

Ivermectin capillary concentrations

No previous studies assessed capillary concentrations of ivermectin; the current study identified a capillary-venous ratio of 1.33 [0.98-1.63] ±29.1%, which was consistent over time from day 2-7 post-treatment (***Table 1***, ***Figure S2***). In the current assays, mosquitoes were fed venous blood via artificial membranes. As capillary concentrations of ivermectin were found to be higher (***Table 1***), and that when mosquitoes bite humans directly they are more likely to feed from capillary blood, the mosquitocidal effects of ivermectin might be higher than presented in the current study. To assess this, an analysis is ongoing comparing mosquito mortality following direct skin feeding (from capillary blood) versus membrane feeding (from venous blood).

Piperaquine PK

Piperaquine pharmacokinetics have been described using meta-analysis, across age-groups, and using capillary sampling, with clearance reported as 55.4 L/h (RSE 4.2%; for an average 54 kg patient),[21] which was somewhat higher in the current study at 97.1 L/h (IIV ±49.1%; for an average 60 kg patient). The majority of previous studies were performed in Asian patients while this study was performed in Kenyan patients. Whilst the current clearance value had a relatively wide percentile range [p5-p95: 20.0-177.3], more studies in African patients might be needed to investigate whether genetics plays a role in piperaquine clearance. The plasma capillary-venous ratio of 1.55 ±36.1% was similar to those reported previously for blood (1.66)[22] and plasma (1.9,[23] 1.63,[24] and 1.41 [25]). Using a linear model, piperaquine's pharmacodynamic effect on QT-interval was also similar to previous studies,[26-29] with the current trial predicting a ΔQTcF-prolongation of 3.71 ms (95% CI: 3.12-4.29) per 100 ng/mL of piperaquine. Additionally, using a dynamic $\boldsymbol{E_{max}}$ model, that simultaneously incorporated piperaquine PK and PD data, we estimated maximal QTcF (449.8 ms) and maximal ΔQTcF (53.5 ms) using population methods (***Table S5*** and ***Figure S5***). Importantly, neither of the two ivermectin regimens used in the study altered piperaquine's pharmacokinetics or dynamics (***Table 1, Figure 2f, Table S4,*** and ***Table S5***).

Drug interaction

It is not possible to completely rule out an effect of DP on ivermectin's pharmacokinetics or pharmacodynamics, as all groups received DP. Nonetheless a substantial drug-drug interaction is unlikely as ivermectin PK parameters were very similar to previously reported values (***Table 1***) and within our prediction, which was based on published data where ivermectin was given alone.[5] Future studies comparing ivermectin alone versus ivermectin with DP could help to further assess any potential effect of DP on ivermectin PK-PD.

Limitations and future studies

A limitation of this PK-PD analysis was that the LLQ for ivermectin was relatively high (venous: 5 ng/mL, capillary: 10 ng/mL), resulting in undetectable concentrations for 34% of venous and 20% of capillary samples in the ivermectin arms post-treatment, predominantly at later timepoints. Previous studies used venous LLQ's of: 0.2,[30] 1.0,[31] 0.5,[7] 2.5,[11] 0.2,[15] 0.8,[32] ng/mL. Based on the number of samples available it was still possible to generate an accurate PK-PD model, however future studies should attempt to use assays with lower LLQ's to make full use of the available samples and detect ivermectin at the very low concentrations that it still has a mosquitocidal effect. Another possible limitation is that this study used a homogeneous laboratory-reared mosquito colony which may not be reflective of wild populations. Although previous work has demonstrated that ivermectin affects survival of all tested anophelines (≥17 species tested),[33] future studies would be beneficial to examine possible (heterogeneity of) effects of ivermectin against wild populations.[8] Finally, this study included only (non-pregnant) adults, whereas the targeted population for MDA also includes children and pregnant women. Children are hypothesized to have faster ivermectin metabolism based on increased CYP3A4 expression.[34] Ivermectin is currently contra-indicated in pregnancy, however inadvertent exposures during MDA for lymphatic filariasis and onchocerciasis have not led

to an increase in adverse events.[35] Further studies assessing the safety, efficacy, and pharmacokinetics of (high-dose) ivermectin are needed in children and pregnant women.

Prolonging mosquitocidal effects

Additional effort is also required to further increase ivermectin's effect-duration past 28 days, as a longer effect-duration would allow MDA rounds to be more widely spaced and reduced in number. Consideration could be given to developing slow-release tablet formulations, to developing gastric-resident devices,[36] or to other similar drugs with a long half-life, such as moxidectin, albeit its LC_{50} of 2,789 ng/mL against *An.gambiae* does not seem to make it very suited for malaria control.[12, 37]

Conclusion

In conclusion, the current pharmacokinetic-pharmacodynamic model predicted that the mosquitocidal effect of high-dose ivermectin lasted for at least 28 days post-treatment, consistent with the in-vivo observations from the main trial.[8] This effect could be explained by ivermectin concentrations alone, without invoking unidentified variables such as an active metabolite or a drug-drug interaction. Furthermore, ivermectin exerted no modification of piperaquine's pharmacokinetics or QTcF-prolongation. The presented ivermectin PK-PD model can be used to design new treatment regimens and predict their mosquitocidal effect. The presented methodology may be useful for the assessment of new endectocides.

Methods

Trial design

Details of the trial design, procedures and main results were published elsewhere.[5, 8] Briefly, the study was a randomized, double-blind, placebo-controlled, parallel 3-arm, superiority trial (ClinicalTrials.gov: NCT02511353). 141 adults with uncomplicated malaria in western Kenya were randomly assigned (1:1:1), stratified by sex and body-mass index, to receive 3 days of ivermectin 600 mcg/kg/day (n=47), 300 mcg/kg/day (n=48), or placebo (n=46), all co-administered with 3 days of DP. The primary outcome was the effect of ivermectin-dose on mosquito mortality. For safety, the effect of ivermectin-dose on piperaquine concentration and piperaquine-induced QTcF-prolongation were also assessed. Venous plasma was collected from day 0 to day 28 using both rich and population sampling strategies. Additional capillary plasma was collected using finger-pricks from day 2+4h (4 hours after the third dose) to day 7, concomitantly with the venous population sampling. Capillary sampling might be useful for subsequent pediatric or field trials and might be more representative of the blood that mosquitoes feed on.

Observed plasma concentrations and outcome data

During 28 days of follow-up, 1,393 venous and 335 paired capillary plasma samples, 850 mosquito-cluster mortality rates (from 91,109 mosquitoes), and 524 QTcF-intervals were collected (***Table S2***). Drug analytical quantification was performed by LC-MS/MS (***Text S1***); only quantifiability and not detectability was considered. Ivermectin venous concentrations were above the lower limit of quantification (LLQ: 5 ng/mL) for 534 samples, of which 246 had paired mortality rates, and capillary concentrations were above LLQ (10 ng/mL) for 181 samples. Piperaquine venous concentrations were above the LLQ (1.5 ng/mL) for 1,246 samples, of which 251 had paired QTcF-intervals, and capillary concentrations were above LLQ (1.5 ng/mL) for 333 samples.

Pharmacokinetic and pharmacodynamic analysis

Pooled exposure-effect analyses were performed using Prism© v7.00. Population pharmacokinetic and pharmacodynamic (PK-PD) modelling and fitting of data were independently performed for ivermectin and piperaquine concentrations using Pmetrics™ v1.5.0.[38] All PK and PK-PD modelling done with Pmetrics™ utilized the non-parametric adaptive grid (NPAG) for finding the non-parametric maximum likelihood estimate of the population distribution. Samples which were below

the limit of quantification were indicated in the Pmetrics data file as "-99" and the lower limit of quantification was incorporated into the overall standard population error model. A proportional scaling factor was used to fit venous and capillary concentrations simultaneously for each drug model (***Supplementary Equations***).

Residual analysis was performed for all predicted PK and PD data in Pmetrics. This included goodness-of-fit comparisons of individual and population predictions against observed data, as well as weighted residuals over time and concentration, to investigate any biases towards higher concentrations or later time points. Visual Predictive Checks (VPCs) were performed using the Pmetrics™ simulator tool, where 1,000 subjects were simulated based on the generated median PK parameters, their variances and correlation matrices as generated in Pmetrics™ for each drug. Additionally, weight variation among patients and associated dosing brackets were included in the simulation. The weight variation was set to match that of the study population and a median of 60 kg patient weight were used for the simulation. The Pmetrics simulator predicted patients' weights for 1,000 patients in the range of 45-101 kg, which is the range of this study. The median, 5% and 95% percentiles with their 95% confidence intervals were plotted against observed data for the VPC graphs. A similar simulation was done using a simultaneous PK-PD model with 1,000 subjects to predict the overall mosquitocidal activity in the study population. To avoid the complexity of adding different dosing brackets to different patient weights, simulations were performed with a standard patient weight only, which is 600 mcg/kg/day or 300 mcg/kg/day administered to 60 kg patients. The wide margins used for CL/F and V/F would account for more variability than that introduced by variability in weight.

Ivermectin PK-PD analysis

Modelling ivermectin was performed using multiple approaches to address several questions about its PK-PD relationship against mosquitoes. As it had been hypothesized that ivermectin's mosquitocidal effects at later timepoints could be due a metabolite or drug-drug-interaction, an initial sequential analysis (using three approaches, see below) was performed to justify a final simultaneous analysis. The sequential analysis, where the PK model is blinded to PD data, assessed the consistency of ivermectin's exposure-effect relationship over time. The simultaneous analysis presumes ivermectin concentrations alone can explain the mosquitocidal activity; this allows for the model to predict best PK and PD parameter estimates using both pharmacokinetic and pharmacodynamic data simultaneously to achieve well-informed parameters that are derived from two sets of data (pharmacokinetic and pharmacodynamic data) rather than parameters being informed by a single set of data (i.e. pharmacokinetic information only, as in the first step of the sequential model).

Sequential modelling approach

For both the sequential and simultaneous approaches, pharmacokinetic analysis was performed using a two-compartment model (***Figure S1***), which displayed a better fit than a one compartment model (***Table S1***). A three-compartment model fit was not attempted for ivermectin as predictive visual checks showed that ivermectin follows a two-compartment model trend and does not display a tri-exponential trend. The two-compartment PK model was performed using ***Equations S1-S13*** (Supp. Materials).

Pharmacodynamic analysis of the relationship between ivermectin concentrations and mosquitocidal effect was performed according to the following $\boldsymbol{E_{max}}$ sigmoidal equation:

$$E = \frac{E_{max} \cdot C}{EC_{50} + C} + E_{min} \quad \text{..... (Eq. 1)}^{39}$$

Where E represents the mosquitocidal effect of ivermectin expressed as the mortality rate of mosquitoes per 100 days, E_{min} is the baseline natural mortality rate, E_{max} is the maximal possible effect relative to E_{min}, EC_{50} the concentration necessary to achieve a rate halfway between baseline and maximal effect (E_{max}+E_{min}), and C is ivermectin concentration in grams/liter.

The sequential PD analysis was performed using three approaches. The first was fitting an E_{max} function through observed mosquito mortality rates and pooled observed concentrations in Prism 7®. The second followed the same methodology using predicted concentrations in order to assess whether (BLQ) concentrations predicted by the Pmetrics™ PK model (blinded to PD data) showed a similar exposure-effect relationship to observed concentrations. The third approach used Pmetrics™ for the population modelling of both PK and PD separately, where each patient in the PD analysis had fixed PK parameters which matched their individual predicted parameters in the PK run. This allowed predicting population variability on PD parameters using the sequential PK-PD model (which cannot be assessed using pooled data in the previous two approaches).

The relationship between ivermectin exposure and its mosquitocidal effect was investigated separately for each day of the study (days: 2+4h, 7, 10, 14, 21 and 28), using predicted PK parameters (second sequential model), as any time-dependent variation could potentially suggest the presence of an unidentified mosquitocidal metabolite.

Simultaneous modelling approach

The simultaneous approach used the same equations above, however all available PD data (ivermectin mosquito mortality rates) were entered into the initial modelling process and both the PK and the PD data for all subjects were modelled simultaneously, allowing for PD data to enhance the PK fit, and vice versa, based on the assumption that ivermectin in its parent form is solely responsible for any increase in the mosquito mortality rate through-out the study duration.

Piperaquine PK

Piperaquine PK was analyzed using a three-compartment model (***Figure S1***) according to the ***Equations S1-S13*** (Supp. Materials). This was compared to one and two compartment models which resulted in poorer fits as evidenced from -2 loglikelihood and AIC and BIC criteria (***Table S1***).

Piperaquine PD

Piperaquine effect upon QTcF-interval was analyzed using two methods: (1) a linear model, according to equation 2 below, which is commonly used in similar published analyses, and (2) an E_{max} sigmoidal model, utilizing previously discussed equation 1, with the effect (E) being QTcF-interval in milliseconds at any given concentration, and C being piperaquine concentration. The data was either analyzed using pooled observed piperaquine concentration versus QTcF-interval (linear model) or simultaneously fitted using a population method with Pmetrics™ as described for the simultaneous ivermectin PK-PD model (E_{max} model).

$$\Delta QTcF = intercept + slope \cdot C \qquad \text{..... (Eq. 2)}$$

The slope represents the relationship between piperaquine concentration in ng/mL and the change in ***QTcF (ΔQTcF)*** in milliseconds.

Secondary PK Parameters Post-hoc Statistical Analysis

$AUC_{0\text{-}28d}$ is defined as the predicted area under the curve for 3 doses of ivermectin or piperaquine over a period of 28 days and was determined using the AUC trapezoidal approximation algorithm in Pmetrics™ for each subject individually. C_{max} represents the maximal predicted concentration for

each subject in the study. T_{max} is the time at which C_{max} is achieved for each individual and terminal $t_{1/2}$ is calculated from the predicted PK slope for each individual between 27-29 days after treatment.

The primary PK parameters for ivermectin $(k_a, Q_1/F, V_{P1}/F, CL/F, V/F)$ and primary PK parameters for piperaquine $(k_a, Q_1/F, V_{P1}/F, Q_2/F, V_{P2}/F, CL/F, V/F)$ and secondary PK parameters for both drugs $(C_{max}, AUC_{0\text{-}28d})$ for each subject in the study were correlated to each other as well as with age, sex, weight, height, QTcF interval (measured at days 0, 2, 2+4h, and 28 after dosing initiation) or mosquito mortality rate (at each study time point). Correlation analysis was done using SPSS® v.24 (IBM Corp®) using Spearman bivariate correlation to identify any possible effects that require further analysis. This included any effect of ivermectin exposure upon piperaquine, exploring any possible interaction between study arm (i.e. ivermectin dose 0, 300, or 600 mcg/kg/day), interaction between all structural parameters (e.g. CL, V, Q_1/F, V_{P1}/F, and k_a), and interaction between observed and predicted C_{max}, and AUC for both drugs. Correlations were considered significant if $\rho>0.3$ and $p<0.05$.

Study Highlights:

What is the current knowledge on the topic?

Ivermectin has been shown, *in-vitro* and *in-vivo*, to kill malaria mosquitoes after feeding on human blood. Previous clinical studies showed an effect for 7 days post-treatment. A recent clinical trial showed a prolonged effect for at least 28 days post-treatment.

What question did this study address?

Using data from the recent trial, this study explored the PK-PD relationship between ivermectin (when co-administered with the antimalarial dihydroartemisinin-piperaquine) and the observed mosquitocidal effects, in order to understand whether an unidentified metabolite or an ivermectin-piperaquine drug interaction could be contributing to the prolonged effect.

What does this study add to our knowledge?

The study shows a time-independent PK-PD relationship between ivermectin exposure in individuals and its mosquitocidal activity, without the need to invoke unidentified variables such as an active metabolite or a drug-drug interaction.

How might this change clinical pharmacology or translational science?

A comprehensive PK-PD model able to predict the mosquitocidal effect of varying ivermectin regimens in this population can be utilized in guiding future studies and mass drug administration for malaria elimination programs.

Declarations:

Acknowledgements

Many thanks to all those that made this study possible, including the study participants and staff, the members of the Trial Steering Committee (Teun Bousema, Kevin Kobylinski, and Brian Foy), and of the Data Monitoring & Ethics Committee (Alejandro Krolewiecki, James Oloo, Timothy Collier, and Carlos Chaccour). Further thanks to the Mundo Sano Foundation and Elea Laboratories for donating the ivermectin, and NeurOptics for donating pupillometers. Finally, we would also like to thank the Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) and the Kisumu County Ministry of

Health (MoH), for hosting the study, Donald Tjia, Ophthalmologist, for his support, and Jacco Veldhuyzen, Emergency Medicine Physician, for assisting in interpreting ECGs. This paper is published with the permission of KEMRI Director. The findings and conclusions in this paper are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention.

Authors' contributions

M.R.S., E.O.O., Da.W., T.K.K., B.O.A., T.B., N.M.B., J.E.G., A.M.S., M.R.D., P.A.P., S.K.K., Du.W., F.O.t.K., S.A.W., and G.A. wrote the manuscript; M.R.S., G.A., E.O.O., S.A.W., and F.O.t.K. designed the research; M.R.S., E.O.O., T.K.K., B.O.A., and Da.W. performed the research; M.R.S. and G.A. analyzed the data; Da.W. and S.A.W. contributed new reagents/analytical tools.

List of abbreviations

AUC	Area under the curve
BLQ	Below Limit of Quantification
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
C_{max}	Maximum drug concentration
CYP	Cytochrome P450
E	Effect of drug on outcome measure (e.g. mosquito mortality rate)
E_{min}	Minimum possible effect (e.g. the effect without exposure to the drug).
E_{max}	Maximal possible effect of the drug relative to E_{min}.
E_{xx}	XX% of effect between E_{min} and E_{max}+E_{min}
EC_{xx}	Concentration at which E_{xx} is achieved.
DHA	Dihydroartemisinin
DP	Dihydroartemisinin-piperaquine
IRS	Indoor Residual Spraying
IVM	Ivermectin
IQR	Inter Quartile Range
JOOTRH	Jaramogi Oginga Odinga Teaching and Referral Hospital
KEMRI	Kenya Medical Research Institute
LC_{50}, unadjusted	Unadjusted Lethal Concentration 50%: within a specified assay duration, the drug concentration killing 50% of all mosquitoes (incl. those that would have died anyway).
LC_{50}, adjusted	Adjusted Lethal Concentration 50%: within a specified assay duration, the drug concentration killing 50% of mosquitoes that normally would have survived (excl. those that would have died anyway).
LC-MS/MS	Liquid chromatography – tandem mass spectrometry
LLIN's	Long-lasting Insecticide Treated Nets
LLQ	Lower Limit of Quantification
LSTM	Liverpool School of Tropical Medicine
MDA	Mass drug administration
MESA	Malaria Eradication Scientific Alliance
MoH	Ministry of Health
PD	Pharmacodynamic
PK	Pharmacokinetic
PPQ	Piperaquine
QTc	Electrocardiogram QT-interval, corrected for heart rate

QTcF	QTc, corrected for heart rate using Fredericia's formula
ΔQTc	QTcF, adjusted for baseline
ΔΔQTc	QTcF, adjusted for baseline and placebo
T_{xx}	Time post-treatment at which EC_{xx} and E_{xx} are achieved.

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Supplementary Materials

1. Text S1
2. Supplementary Equations
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Figure Legends

Figure 1 Ivermectin pharmacokinetic model (sequential approach) using venous and capillary concentrations: goodness-of-fit and simulation. **(a)** Ivermectin individual predicted concentrations (n=1029) versus observed concentrations (n=708) ($slope$=0.98, R^2=0.8652). **(b)** Ivermectin population predicted concentrations versus observed concentrations ($slope$=0.81, R^2=0.5793). **(c)** Weighted residual error distribution of predicted versus observed ivermectin concentrations over time (mean=-0.23 over 28 days) (dashed black line= LOESS curve fit through residuals). **(d)** Weighted residual error distribution of predicted versus observed ivermectin concentrations over predicted ivermectin concentration (mean=-0.23 over a range of 1 to 353 ng/mL) (dashed black line= LOESS curve fit through residuals). **(e)** Observed ivermectin venous concentrations (grey circles) with predicted concentrations for those unobserved (gray squares), overlaid with simulation of ivermectin 300 mcg/kg/day for 3 days (solid black line= median; dashed grey lines= 5% and 95% percentiles; shaded grey area= 95% CI for the percentiles). **(f)** Similar to (e) with ivermectin 600 mcg/kg/day for 3 days. IVM=ivermectin. Conc.=concentration. LLQ=lower limit of quantification, 5 ng/mL (horizontal grey line). Simulations included 1,000 individuals of 60 kg bodyweight.

Figure 2 Piperaquine pharmacokinetic model using venous and capillary concentrations: goodness-of-fit and simulation. **(a)** Piperaquine individual predicted concentrations (n=1581) versus observed concentrations (n=1578) ($slope$=1.04, R^2=0.9273). **(b)** Piperaquine population predicted concentrations versus observed concentrations ($slope$=0.93, R^2=0.8332). **(c)** Weighted residual error distribution of predicted versus observed piperaquine concentrations over time (mean=-0.23 over 28 days) (dashed black line= LOESS curve fit through residuals). **(d)** Weighted residual error distribution of predicted versus observed piperaquine concentrations over predicted ivermectin concentration (mean=-0.20 over a range of 2 to 1421 ng/mL) (dashed black line= LOESS curve fit through residuals) **(e)** Observed piperaquine venous concentrations (grey circles) overlaid with simulation of piperaquine 960 mg/day for 3 days (solid black line= median; dashed grey lines= 5% and 95% percentiles; shaded grey area= 95% CI for the percentiles). **(f)** Simulation of piperaquine 960 mg/day for 3 days based on parameters derived from patients concomitantly receiving ivermectin 0 mcg/kg/day (solid black line), ivermectin 300 mcg/kg/day (solid grey line) or ivermectin 600 mcg/kg/day (black dashed line). PPQ=piperaquine. Conc.=concentration. LLQ=lower limit of quantification, 1.5 ng/mL (horizontal grey line). Simulations included 1,000 individuals of 60 kg bodyweight.

Figure 3 **(a)** Relationship between observed ivermectin venous concentration and mosquito mortality rate (/100 days). Open circles (n=246 concentrations above LLQ with paired mortality rate) represent observed data. The solid line represents sigmoidal three-parameter E_{max} fit. **(b)** Similar to (a), however now overlaid with predicted ivermectin venous concentrations for all samples (including those that were below LLQ) with observed mosquito mortality rates in patients that received ivermectin (grey squares, n=567). The dashed line represents the sigmoidal three-parameter E_{max} fits for the predicted concentrations. **(c)** A comparison between the exposure relationship of (a), (b) and the exposure-effect relationship generated using the simultaneous PK-PD model which incorporated PD data in the process of PK modelling and vice versa.

Figure 4 The exposure-effect relationship between predicted ivermectin concentrations (from the sequential PK model, using PK data from all days) and corresponding observed mosquito mortality rates separated by day of analysis after initiation of treatment. E_{min} and E_{max} were fixed to the values determined by analyzing the entire dataset and EC_{50} concentrations (95% CI's) were estimated as shown in the figure.

Figure 5 Ivermectin pharmacokinetic and pharmacodynamic simulation of mosquito mortality rate (using simultaneous approach) with **(a)** 300 mcg/kg/day for 3 days, and **(b)** 600 mcg/kg/day for 3 days. Mosquito mortality rate simulated median (solid black line), 5th and 95th percentiles (dashed lines), and 95% confidence intervals (shaded grey areas), with observed mosquito mortality rates per sample (open circles), observed median±IQR mortality rate per study visit (ball-whiskers), and E_{min} (horizontal dashed line). **(c)** Comparison of both regimens with a simulation of the standard 150 mcg/kg single-dose. Simulations included 1,000 individuals of 60 kg bodyweight. **(d)** Mortality rate ratios calculated as incidence rate ratios using the PK-PD model (IRR; lines) and as hazard ratios with 95% confidence intervals as per main efficacy results[8] (HR; triangles: 600 mcg/kg/day for 3 days versus placebo, squares: 300 mcg/kg/day for 3 days versus placebo, and whiskers: 95%CI's).

Table 1 *Primary and secondary PK parameters for ivermectin and piperaquine. Ivermectin parameters are reported using either the sequential PK-PD model (where PK analysis was done on venous and capillary PK data only) or the simultaneous PK-PD model (where PK-PD analysis was performed simultaneously on venous and capillary exposure data as well as PD outputs defined as corresponding mosquito mortality rates for each venous sample). WT=bodyweight. NA=not available. *For ivermectin models, only subjects in the ivermectin arms.*

Parameter	**IVERMECTIN** **Sequential PK-PD model** **Median** **[p5-p95]** ± **Inter-individual variability (%)**	**IVERMECTIN** **Simultaneous PK-PD model** **Median** **[p5-p95]** ± **Inter-individual variability (%)**	**PIPERAQUINE** **Median** **[p5-p95]** ± **Inter-individual variability (%)**
V / F (L)	147.0 [36.2-582.0] × (WT/60) ±103.0	161.7 [70.9-760.4] × (WT/60) ±112.7	803.7 [149.1-1999] × (WT/60) ±69.0
CL / F (L/h)	9.6 [6.5-14.6] × $(WT/60)^{0.75}$ ±39.1	10.9 [6.6-26.1] × $(WT/60)^{0.75}$ ±57.4	97.1 [20.0-177.3] × $(WT/60)^{0.75}$ ±49.1
k_a (h^{-1})	0.22 [0.082-1.79] ±157.1	0.474 [0.15-6.93] ±163.5	1 (fixed)
Q_1 / F (L/h)	19.0 [7.7-113.1] ±104.7	21.1 [9.7 – 116.4] ±100.5	1017.0 [197.5-4079] ±87.5

V_{P1} / F (L)	1148.1 [413.1-3845] ±86.6	612.4 [253.0-1879] ±82.1	3796 [415.1-14918] ±165.8
Q_2 / F (L/h)	NA	NA	156.4 [32.6-545.7] ±82.8
V_{P2} / F (L)	NA	NA	35993 [7586-146968] ±96.2
Capillary/venous ratio	1.32 [1.1-1.6] ±18.7	1.33 [0.98-1.63] ±29.1	1.55 [1.1-2.8] ±36.1
	Secondary parameters		
C_{max} (ng/mL)			
All subjects	NA	NA	252.4 [95.5-1072.5]
0 mcg/kg/day IVM arm	NA	NA	250.1 [99.4-727.7]
300 mcg/kg/day IVM arm	64.1 [29.7-129.9]	69.4 [34.1 -196.3]	263.6 [84.6-1268.2]
600 mcg/kg/day IVM arm	105.2 [44.5-482.5]	118.9 [45.2-455.1]	246.2 [98.8-990.1]
T_{max} (h) – after last dose			
All subjects*	4.8 [0.58-8.7]	2.9 [0.46-7.8]	1.4 [1.1-3.6]
0 mcg/kg/day IVM arm	NA	NA	1.4 [1.1-3.5]
300 mcg/kg/day IVM arm	5.0 [1.4-8.8]	3.9 [0.75-7.6]	1.4 [1.1-3.0]
600 mcg/kg/day IVM arm	3.5 [0.40-7.5]	2.3 [0.43-8.2]	1.5 [1.1-3.8]
Terminal $t_{1/2}$ (h)			
All subjects*	4.9 [1.9 – 12.9]	3.1 [0.93-11.4]	17.7 [2.6-81.8]
0 mcg/kg/day IVM arm	NA	NA	18.2 [5.7-89.8]
300 mcg/kg/day IVM arm	5.1 [2.2-12.6]	2.9 [1.1-7.8]	17.2 [2.8-94.6]
600 mcg/kg/day IVM arm	4.7 [1.7-12.5]	3.2 [0.90-8.5]	17.9 [1.5-42.3]
$AUC_{0\text{-}28d}$ (h • mcg/mL)			
All subjects	NA	NA	21.4 [6.3-47.9]
0 mcg/kg/day IVM arm	NA	NA	21.3 [10.4-44.9]
300 mcg/kg/day IVM arm	5.5 [2.5-8.3]	5.0 [1.6-8.3]	23.7 [6.9-49.7]
600 mcg/kg/day IVM arm	10.0 [1.7-22.3]	9.3 [2.1-25.0]	21.2 [6.5-46.5]

Table 2 *Pharmacodynamic parameters of ivermectin as determined using (1) observed venous concentrations only, (2) predicted venous concentrations from the sequential PK model, or (3) using a simultaneous PK-PD model which incorporates PK and PD data in the analysis process. E_{min} was fixed to 3.7 for (1) and (2) based on baseline and control mortality. E_{xx} is the percentile of effect between E_{min} and $E_{min}+E_{max}$. EC_{xx} is the concentration at which E_{xx} is achieved. T_{xx} is the time post-treatment at which EC_{xx} and E_{xx} are achieved.*

Models based on:	**Obs. Venous Conc. versus Mosq. Effect (Pooled Data)**	**Sequential Pred. Venous Conc. versus Mosq. Effect (Pooled Data)**	**Simultaneous PK-PD model (Population Modelling)**
Parameter	**Median [p5-p95]**	**Median [p5-p95]**	**Median [p5-p95]**
Mosquitocidal effect			
E_{min} (deaths/100 days)	3.7 (fixed)	3.7 (fixed)	3.9 [2.9-5.8]
E_1 (deaths/100 days)	4.18 [4.16-4.21]	4.22 [4.19-4.25]	4.40 [4.23-4.53]
E_5 (deaths/100 days)	6.14 [6.02-6.25]	6.31 [6.18-6.47]	6.38 [5.54-7.08]
E_{50} (deaths/100 days)	28.1 [23.2-29.3]	29.9 [28.5-31.4]	28.7 [20.1-35.5]
E_{95} (deaths/100 days)	50.1 [47.7-52.3]	53.4 [50.8-56.2]	50.7 [34.9-64.1]
E_{max} + E_{min} (deaths/100 days)	52.5 [50.0-54.9]	56.0 [53.3-59.0]	53.4 [36.7-67.5]
Effective Concentration			
EC_1 (ng/mL)	0.11 [0.095-0.14]	0.17 [0.14-0.19]	0.16 [0.053-0.37]
EC_5 (ng/mL)	0.90 [0.51-1.14]	0.84 [0.74-1.0]	0.83 [0.28-1.9]
EC_{50} (ng/mL)	11.3 [9.5-13.6]	16.5 [14.1-19.2]	15.9 [5.3-36.4]
EC_{95} (ng/mL)	214.3 [178.2-259.0]	314 [269.3-374.3]	302.1 [100.7-691.6]
Time above EC (IVM-3x300)			
T_1 (days)	33.3 [19.0-130.5]	30.1 [17.9- 115.4]	24.4 [10.5-63.0]
T_5 (days)	22.6 [13.4-56.4]	20.3 [12.2-50.9]	17.0 [8.0-42.6]
T_{50} (days)	5.6 [2.4-12.7]	3.9 [2.2-8.9]	4.1 [NA-8.1]
T_{95} (days)	NA	NA	NA
Time above EC (IVM-3x600)			
T_1 (days)	38.0 [21.6-150.1]	34.5 [20.0-142.2]	28.1 [11.5-72.8]
T_5 (days)	27.4 [15.4-66.0]	24.8 [14.3-60.4]	20.1 [9.3-52.9]
T_{50} (days)	9.0 [3.0-20.7]	7.1 [2.6-16.4]	6.8 [2.5-14.7]
T_{95} (days)	NA	NA	NA