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The repurposing of ivermectin for malaria: a prospective pharmacokinetics-based virtual clinical trials assessment of dosing regimen options

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ABSTRACT

Ivermectin has demonstrated many successes in the treatment of a range of nematode infections. Considering the increase in malaria resistance attention has turned towards ivermectin as a candidate for repurposing for malaria. This study developed and validated an ivermectin physiologically-based pharmacokinetic model in healthy adults (20-50 years) and paediatric (3-5 years/15-25 kg) subjects and in a representative adult malaria population group (Thailand). Dosing optimisation demonstrated a twice daily for 3- or 5-day regimens would provide a time above the LC50 of more than 7 days for adult and paediatric. Furthermore, to address the occurrence of CYP450-induction often encountered with antiretroviral agents, simulated drug-drug interaction studies with efavirenz highlighted that a 1 mg/kg once daily dose for five days would counteract the increased ivermectin hepatic clearance and enable a time above LC50 of 138.8 hours in adults and 141.2 hours in paediatric subjects.

It was also demonstrated that dosage regimen design would require consideration of the age-weight geographical relationship of the subjects, with a dosage regimen for a representative Thailand population group requiring at least a single daily dose for 5 days to maintain ivermectin plasma concentrations and a time above LC50 similar to that in healthy adults.

KEYWORDS

Physiologically-based pharmacokinetics; pharmacokinetics; drug resistance; Onychomycosis; *in-silico* modelling.

1. INTRODUCTION

In May 2015, the World Health Organisation published a future strategy for tackling malaria, the 'Global Technical Strategy for Malaria 2016-2030'[1], which highlighted the need for continued work towards tackling the significant risks many of the world's population face with malaria infection. It has been estimated that 3.2 billion people are at risk of malaria with up to 283 million cases of diagnosed malaria worldwide in 2013. Despite a global decline in malaria mortality rates, there still remain challenges in many regions, particularly within sub-Saharan Africa, where the greatest level of mortality is evident [1]. Where effective drug treatments are available, the mortality that is associated with treatments for falciparum malaria is < 0.1 % [2]. However, where the parasite is able to multiply untamed, the parasite burden of the host increases resulting in organ dysfunction, impairment of higher brain function, loss of consciousness and anaemia, culminating in death.

A major shift in treatment strategies for malaria may be required, considering the increasing prevalence of anti-microbial resistance which has been exemplified by the emergence of resistance to chloroquine [3] and sulfadoxine/pyrimethamine (SP) [4,5]. Furthermore, the appearance of an artemisinin drug-resistance strain of malaria within the Greater Mekong Subregion (GMS), first identified in Cambodia in 2008 [6], poses particular concerns and demonstrates the need to explore alternative antimalarial agents. This is further highlighted by the increasing treatment failure associated with mefloquine and piperaquine across the GMS [7-10].

The 'Global Technical Strategy for Malaria'[1] comments on novel approaches required to aid malaria treatment and specifically focuses on opportunities for 'innovation in medicines', which provides a framework for the acceleration of malaria elimination. Given the complexity of current drug discovery and development strategies, consideration of existing, clinically approved, candidate molecules with a view to repurposing for malaria has many advantages. For example, the safety profile and clinical pharmacokinetics would have been established, and fast-track processes (e.g. FDA) allow for the establishment of new clinical indications [11]. Such approaches have found successes in the area of orphan diseases [12], where specific unmet need exists and where traditional drug development strategies would be time-consuming. Ivermectin, is one potential candidate that may be suited for repurposing to malaria. Ivermectin is an endectocide and kills a range of parasites and associated vectors, and is currently marketed and licenced to treat onchocerciasis, lymphatic filariasis,

strongyloidiasis, scabies and head lice [13]. The use of ivermectin in the treatment of onchocerciasis has been well documented over the past 25 years with community-wide mass drug administration (MDA) of ivermectin contributing to the near elimination of onchocerciasis [14]. Further, numerous studies have demonstrated that ivermectin can remain in the blood stream for a sufficiently long time-frame, following standard dosing, to kill the *Anopheline* vector [15-19] and malaria parasite [20].

The importance of ivermectin as a potential novel drug for repurposing to malaria is exemplified by the formation of the 'Ivermectin Research for Malaria Elimination Network' [21], whose primary goal was to establish a common research agenda to aid in the generation of evidence base on which to support (or otherwise) whether ivermectin should be repurposed to malaria. Further we strongly recommend those wishing to gain an in-depth understanding of the repurposing ivermectin to consider a recent series of reviews exploring the pharmacokinetics evidence, regulatory policies and clinical development pathways to support the repurposing of ivermectin to malaria [22-24]

Ivermectin shows rapid absorption with an absorption half-life of 0.5-2.5 hours [25,26], is highly lipophilic and is associated with extensive protein binding (fu < 0.1) and a large volume of distribution (3.1-3.5 L/kg) [25]. The metabolism of ivermectin is primarily mediated by Cytochrome P450 3A4 (CYP3A4) [27] and leads to a half-life of approximately 18 hours [25]. A complete description of the pharmacokinetics of ivermectin can be found in two review publications [22,25].In clinical studies, ivermectin has been used across an extensive dosing range, with over 2.7 billion single doses of the 0.15-0.2 mg/kg dose administered through the Mectizan Donation program [28] as single doses. Furthermore, higher doses of up to 2 mg/kg as single doses have been administered [29] whilst the Centre for Disease Control and Prevention have recommended doses of up to 1.4 mg/kg for severe crusted scabies [30]. The wide safety profile would suggest higher doses are well tolerated. However, there are no current clinical trials assessing possible dosing regimens that could be used to identify an appropriate treatment regimen for use in malaria. A recent report has identified the ivermectin concentration capable of killing 50 % (LC50) mosquitoes as being approximately 16 ng/mL [31], which could be used as a first-principle potential target concentration for 'therapeutic-effect'.

This manuscript, therefore, attempts to pragmatically assess the impact of possible dosing regimen designs on ivermectin plasma concentrations, with an emphasis on maintaining

plasma concentration above the LC50, through the application of physiologically-based pharmacokinetic (PBPK) modelling using virtual clinical trials.

The key objectives were therefore to: (i) assess the impact of dose escalation of ivermectin on adults (20-50 years old) and paediatrics within the age range of 3-5 years old, who pose significant challenges in treatment and are prone to developing severe malaria [1]; (ii) given that ivermectin is metabolised by CYP3A4, to assess the impact of induction based drug-drug interactions (DDIs) on reducing ivermectin plasma concentrations in adults and children and (iii) to illustrate the potential changes in ivermectin pharmacokinetics when dosed to a representative malaria population group originating from the GMS (i.e. Thailand).

2. METHODS

All population based PBPK modelling was conducted using the virtual clinical trials simulator Simcyp (Simcyp Ltd, a Certara company, Sheffield, UK, Version 16). Unless otherwise stated, mixed gender (50:50) populations were simulated. A six-stage workflow approach was applied for the development, validation and simulation of the ivermectin (Figure 1). The default Simcyp validated adult and paediatric 'healthy volunteer' population groups were used in simulations for Steps 1-5. The latter population group accounted for ontogenic related changes in physiological/biochemical parameters such as organ volumes, organ perfusion and drug metabolising enzymes [32-34]. Further, the Simcyp population groups account for population variability through the inclusion of a variability metric (% coefficient variability) which was established from public health data bases such as the US National health and Nutrition Examination Survey (https://www.cdc.gov/nchs/nhanes/).

2.1 Step 1: Base model development and validation

A full description of the model development can be found in Section 1 of the Supplementary Materials. For model development, clinical studies selected included: (i) single doses (30, 60, 90 and 120 mg) and multiple doses (30 mg and 60 mg daily for 7 days) in healthy subjects [29]; (ii) a single (tablet) 12 mg dose administered to healthy subjects [35]; (iii) a single 0.15 mg/kg dose administered to healthy subjects [36]; (iv) a single 0.20 mg/kg dose administered to healthy subjects [37]; a single 0.15 mg/kg dose administered to healthy subjects [38]; (v) single 0.15 mg/kg dose administered to onchocerciasis subjects [39]. A recent study by Ouédraogo et al. 2015 [40] provided some additional ivermectin plasma concentration data, but this was excluded from the validation approaches due to the sparse nature of the data and the

lack of quantitative summary pharmacokinetics data (e.g C_{max}, t_{max} and AUC) with which to directly compare.

Model development and refinement was conducted using the single and multiple doses studies in healthy subjects reported by Guzzo *et al.* (2002) [29] (clinical study (i) as detailed above). Model validation was subsequently assessed against clinical studies ii-v (as detailed above). In all cases, model simulations were run to match the reported age range and subject number as reported by each study.

Final ivermectin compound parameters that were applied to all subsequent steps are detailed in table 1, with the supplementary materials (Section 1) fully describing the approaches used to determine these parameter values.

2.2 Step 2: Adult escalating dose study

Previous ivermectin clinical studies have used single doses of between 1.4-2 mg/kg [29 30], and therefore to define a potential upper limit of the therapeutic window, a single oral dose of 2 mg/kg was administered using a Simcyp predefined healthy volunteer population with 100 subjects. The upper therapeutic window band was estimated from the mean maximum concentration within the population group with the lower band set at the LC50 (16 ng/mL) [31].

Subsequently, simulations were run using the healthy volunteer population aged 18-50 years (100 subjects) with ivermectin dosed orally at 0.15, 0.3 and 0.6 mg/kg as a single daily dose. Thereafter, the dose resulting in the greatest time above the LC50 (but below the upper limit of the therapeutic window) was selected and assessed under 3-day dosing and 5-day dosing, each with dosing intervals (τ) of 12- or 24-hours, representing dosing regimens that are widely used for common antimalarials such as artemether, lumefantrine and piperaquine [31]. Finally, the dosing regimen resulting in the greatest time above the LC50 was selected as the optimal dosing regimen in adults.

2.3 Step 3: Paediatric escalating dose study

Simulations were run using the Simcyp paediatric population group and designed to ensure simulations contained at least 100 subjects aged 3-5 years old and covering weight bandings of 15-25 kg. Dose escalation regimens were based on the optimal dose identified in adult population groups (Step 2) with the dosing regimen resulting in the greatest time above the LC50 selected as the optimal dosing regimen in paediatrics (healthy volunteer populations).

2.4 Step 4-5: Impact of induction-based drug-drug interactions on dosing strategies

Many malaria patients are often co-infected with other communicable diseases such as HIV [41]. In these cases, the pharmacotherapy requirements are often complex with multiple competing drug-drug interactions (DDIs) possible. Antiretroviral agents such as efavirenz have been demonstrated to induce the expression of CYP3A4 [42-44] and subsequently increase the metabolic clearance (and hence reduce plasma concentrations) of antimalarial agents [45-48] [49]. This may potentially increase the risk of malaria recrudescence and place the patients at risk of developing severe malaria. Therefore the potential risk of CYP3A4 induction on reducing the plasma concentration of ivermectin was assessed in this step.

Dosing strategies utilised weight-based dosing for adults (Step 4) and children (Step 5), with simulations run for between 15-21 days [50,51] with efavirenz dosed throughout the study duration (Adults: 600 mg once daily; Paediatrics: 250 mg once daily for 15 kg to < 20 kg and 300 mg once daily for 20 kg to < 25 kg) and ivermectin dosing initiated at day 13, to ensure stable induction of CYP3A4 prior to ivermectin dosing. The impact of DDI was assessed through changes in the time above the LC50.

2.5 Step 6: Ivermectin dosing in a 'malaria-type' population group

To assess the impact of a potentilal changes in ivermectin pharmacokinetics when dosed in a non-Caucasian/Malaria infected population group, we utilised an Asian (Thailand) population group that was developed in a previous publication by our group to assess antimalarial pharmacokinetics within a malaria-infected population group [51]. This Thai population group was adapted to include appropriate geographical age-weight distributions for male and female adults and paediatrics. These adaptations also included revised blood biochemistry to match patient demographics identified within malaria patients. The development of this Thailand popualtion group is fully described in the Supplementary Materials (Section 2). Simulations were performed based on optimal doses identified in previous sections.

2.6 Predictive performance

In all of the validation simulations (Step 1), predictions within 2-fold of the observed data were generally considered to represent an 'optimal' predictive performance and confirmed successful model development and validation, despite there being no uniform standard of acceptance to determine this criterion [52-54]. This 2-fold acceptance criterion was subsequenlty

utilised in comparisons of simulated plasma-concentration profiles with published clinical data, where reported.

2.7 Data and statistical analysis

The observed data from clinical studies that were used for visual predictive checks were extracted using WebPlotDigitizer v.3.10 (http://arohatgi.info/WebPlotDigitizer/). Unless otherwise stated, all simulations employing weight-based dosing were run with 100-subject simulation in a 10x10 trial (10 subjects per trial with 10 trials) to account for reasonable inter-/intra individual variability being captured within the model simulations. Where necessary, pooling and post-processing of output Simcyp data were conducted to match individuals to the required age-weight boundary conditions for the study.

Where a DDI was simulated, the model performance was principally dictated by the comparison of the AUC ratio or C_{max} ratio (ratio of the AUC or C_{max} in the absence and presence of the efavirenz). An AUC ratio or C_{max} ratio greater than 1.25 is indicative of an inhibition reaction whereas a ratio of less than 0.8 indicating an induction reaction whilst a ratio of between 0.8 – 1.25 indicating no interaction. Where applicable, statistical analysis was conducted using paired t-tests with a $P < 0.05$ indicating statistical significance.

3. RESULTS

3.1 Step 1: Validation

An ivermectin compound file was developed within Simcyp and validated against a range of published studies using the healthy volunteer population group. Model development considered a range of single [29 35] and multi-dose studies coupled with more traditional weight based dosing (0.15-0.20 mg/kg) [36-39], and in all cases simulated ivermectin plasma concentration profiles were within the observed range for each study (Figure 2). Furthermore, the model predicted t_{max}, C_{max} and AUC were predicted to within 2-fold of the reported parameters for each study (Table 2) and confirmed successful model validation.

However, model predicted AUC_{0-t} (AUC calculated from the study duration time only) was 3.9-fold underpredicted when compared to the study by Baraka *et al* (1996) [36] (Table 2). In contrast, model predicted AUC was within 2-fold when compared to that reported in the

study Okonkwo *et al* (1993) [39] for the same dose as that utilised by Baraka *et al* (1996) [36] (Table 2).

3.2 Step 2: Adult escalating dose study

Simulations were next performed to assess the impact of dosing-escalation on the time above the suggested LC50 (16 ng/ml). Single dose studies across a dosing range of 0.15-0.6 mg/kg (Figure 3A) resulted in a C_{max} above the LC50 for all subjects, with higher doses resulting in a longer duration of time above the LC50, 10.4 hours for 0.15 mg/kg to 23 hours for 0.6 mg/kg (Table 3). A further dose of 2 mg/kg resulted in a C_{max} of 178.38 ± 95.98 ng/mL (Figure 3B) with a duration of time above the LC50 of greater than 24 hours (Table 3). Based upon the 2 mg/kg dose, the upper 'limit' of the therapeutic window was set at 435.30 ng/mL.

Under repeated daily dosing (once daily for 3 days), a similar trend of increasing time above the LC50 with an increasing dose (Table 3) was observed (Figure 3C). The 0.6 mg/kg dose resulted in time above the LC50 of 152.9 hours (Table 3). Extension of the dosing duration for the 0.6 mg/kg dose from a single daily dose for 3 days, to a twice daily for 3 days (Figure 3D) and twice daily dose for 5 days (Figure 3F) resulted in a significant increase in C_{max} ($P < 0.001$) and time above LC50 (151.51 ± 66.22 ng/mL and 178.24 hours to 174.41 ± 73.69 ng/mL and 257.19 hours) compared to once daily dosing (Table 3).

3.3 Step 3: Paediatric escalating dose study

Simulations were next performed in healthy paediatric population groups aged 3-5 years to assess the impact of a dosing-escalation on the time above the suggested LC50 (16 ng/ml). As with adult populations, single dose studies across a dosing range of 0.15-0.6 mg/kg (Figure 4A) resulted in a C_{max} above the LC50 which was dose dependant and resulted in a longer duration of time above the LC50, 10.1 hours for 0.15 mg/kg to 23.9 hours for 0.6 mg/kg (Table 4). With a higher dose of 2 mg/kg, a C_{max} of 348.40 ± 148.95 ng/mL was simulated (Figure 4B) which remained above the LC50 for greater than 24 hours (Table 4). Based upon a 2 mg/kg dose, the upper 'limit' of the therapeutic window was set at 516.91 ng/mL.

Repeated daily dosing (once daily for 3 days), resulted in a similar trend of increasing time above the LC50 (Figure 4C) (Table 4) with the largest dose (0.6 mg/kg) resulting in a time above the LC50 of 151.2 hours (Table 4).

Upon extension of the dosing regimen from once daily for 3 days to either twice daily for 3 days (Figure 4D), once daily for 5 days (Figure 4E) or twice daily for 5 days (Figure 4F), a significant increase in C_{max} ($P < 0.001$) and time above LC50 compared to once daily dosing (Table 4) was simulated. The longest duration above the LC50 was determined for the twice daily 0.6 mg/kg dose for 5-days, 290.1 hours (Table 4).

3.4 Step 4: Impact of induction-based drug-drug interactions on dosing strategies: adults

To address the potential impact of malaria recrudescence in complex pharmacotherapy, e.g. HIV-coinfection, a DDI was simulated in the presence of the CYP3A4 inducer efavirenz, where the ivermectin dose was escalated. To ensure stable induction of CYP3A4, efavirenz was dosed throughout the simulation period with ivermectin dosing commencing on day 13 onwards. Furthermore, dosing was conducted in such a fashion to ensure the ivermectin C_{max} did not go beyond the upper therapeutic window identified in step 2.

For single daily doses, the impact of efavirenz on ivermectin pharmacokinetics generally resulted in an approximate 50 % decrease in ivermectin C_{max} (Figure 5A) (C_{max} ratio: 0.48) (Table 5) across all doses (0.15 mg/kg to 2 mg/kg) ($P < 0.001$), with the highest dose resulting in a C_{max} of 120.39 ng/mL ± 61.70 ng/mL. Furthermore, the exposure of ivermectin in subjects was also significantly decreased (Figure 5A) with an approximate 75 % decrease in the AUC for across all doses (AUC ratio = 0.28) when compared to the absence of efavirenz ($P < 0.0001$). The time above the LC50 compared to ivermectin alone (Table 3) was also significantly reduced for all equivalent doses ($P < 0.001$), for example when comparing the 0.3 mg/kg dose daily for three days in the absence of efavirenz (time above LC50=86.2 h) (Table 3) to in the presence of efavirenz (time above LC50=19.7 h) (Table 5).

When dosing for 3-days (Figure 5B) or 5-days (Figure 5C), τ=12 hours, the C_{max} was moderately higher than equivalent single daily doses, however an increase in the AUC was simulated which resulted in a significantly higher time above the LC50 for 3-days (1 mg/kg: 77.3 hours; 2 mg/kg: 91.2 hours) or 5-day regimens (1 mg/kg: 138.8 hours; 2 mg/kg: 144.7

hours) compared to a single daily dose for three days (1 mg/kg: 30.8 hours; 2 mg/kg: 47.5 hours) (Table 5) (P<0.001).

3.5 Step 5: Impact of induction-based DDIs on dosing strategies: paediatrics

The induction effects of efavirenz on CYP3A4 metabolism was further assessed in paediatric subjects, aged 3-5 years and spanning two efavirenz dosing bands (250 mg for 15 kg to < 20 kg) and 300 mg for 20 to < 25 kg).

For single daily doses, efavirenz exposure resulted in an approximate 57 % decrease in ivermectin C_{max} (Figure 6A) (C_{max} ratio: 0.43) (Table 6) across doses of 0.6, 1 and 2 mg/kg, with the highest dosing regimen (2 mg/kg for three days) resulting in a C_{max} of 240.45 ng/mL ± 150.97 ng/mL. This was accompanied by an approximate 79 % decrease in the AUC across all doses (AUC ratio = 0.21) when compared to the absence of efavirenz. (Figure 6A) (P<0.001). Furthermore, the time above the LC50 compared to ivermectin alone (Table 4) was also significantly reduced (P<0.001), e.g. comparing the 0.60 mg/kg dose daily for three days in the absence of efavirenz (time above LC50=151.2 h) (Table 4) to in the presence of efavirenz (time above LC50=27.8 h) (Table 6).

When dosing twice daily for 3-days (Figure 6B) or 5-days (Figure 6C), the simulated C_{max} was moderately higher (but not statistically significant) than the equivalent single daily doses (Table 6). This was however accompanied by an increase in the AUC which resulted in a significantly higher time above the LC50 for dosing of twice daily for 3-days (1 mg/kg: 81.2 hours; 2 mg/kg: 104.2 hours) or twice daily for 5-day regimens (1 mg/kg: 141.2 hours; 2 mg/kg: 142.2 hours) compared to a single daily dose for three days (1 mg/kg: 30.9 hours; 2 mg/kg: 30.9 hours) (Table 6).

3.6 Step 6: Ivermectin dosing in a 'malaria-type' population group

Although model simulations have been conducted in a healthy volunteer population group, which broadly follows demographic trends in the Caucasian population, the final stage of the modelling process considered the dosing of ivermectin within a non-Caucasian population group, using a custom designed Thailand malarial adult and paediatric population groups which was previously developed and applied to similar malaria modelling approaches by our group [51], which had appropriate age-weight distributions and associated alterations to blood biochemistry. Ivermectin was dosed at 0.6 mg/kg once daily for three days to adult (Figure 7A) and paediatrics (Figure 7B). A noticeably lower ivermectin plasma concentrations were

simulated for the Thailand population group compared to the healthy volunteer group (Figure 7A) with a similar C_{max} for each dose. However, the time above LC50 was significantly reduced in the Thailand population compared to the healthy volunteer population ($P<0.001$) (152 hours to 67.3 hours). This was however recoverable when the dosing regimen was increased to 1 mg/kg and duration extended to once daily for 5 days, resulting in a C_{max} of 176.12 ng/L ± 82.22 ng/mL and AUC of 4155.15 ng/mL.h ± 2230.82 ng/mL.h. Furthermore, the time above LC50 was 171.6 hours.

The total oral clearance for ivermectin increased from 39.12 L/h ± 21.54 L/h for the Caucasian healthy adults to 45.2 L/h ± 27.41 L/h for the Thailand subjects.

For paediatric subjects, the ivermectin plasma concentration profiles were general similar between Thailand and Caucasian healthy subjects, with a Thailand subjects showing a slightly lower time above LC50, 137.2 hours compared to Caucasian healthy subjects, 154.8 hours (Figure 7B).

4. DISCUSSION

The eradication of malaria has been successful in many countries through the use of artemisinin-based combination therapy (ACT) [1]. However, this optimism has recently been tempered by the appearance of artemisinin–resistance *Plasmodium falciparum* strains in the GMS [7-10]. Despite the urgent need for new antimalarial agents to tackle this increasing risk of resistance, the time-lag associated with the discovery/development and clinical assessment of new drugs precludes the imminent regulatory approval of pipeline candidates [55]. However, drug repurposing provides an approach whereby existing licenced drugs can be 'transferred' to an alternative (proven) indication, thereby bypassing the need for traditional discovery/development pipelines. Such approaches have indeed been useful in repurposing thalidomide to treat multiple myeloma [56] and crizotinib [57] for anaplastic lymphoma kinase gene–rearranged non-small cell lung cancer.

Recent reports have highlighted ivermectin as a potential candidate for repurposing towards malaria [22-24]. Ivermectin is a dihydro derivate of avermectin and was initially licenced for use in veterinary medicines, but has demonstrated immense success in the treatment of Onchocerciasis in addition to a range of other nematode infections including Ascariasis, filariases, Gnathostomiasis and Trichuriasis [58]. Further, reports have also highlighted how ivermectin can remain in the blood stream for a sufficiently long time-frame to kill the *Anopheline* vector [15-19] and malaria parasite [20]. A key advantage of ivermectin therapy is that,

given its wide scale global use with many decades of monotherapy, there is yet to be confirmed scenarios of ivermectin resistance, leading to calls for ivermectin to be given consideration for other potential communicable diseases [22-24,59].

The primary aim of this study was to explore the possible use of ivermectin dosing in adult and paediatric subjects using PBPK modelling through virtual clinical trials analysis. Such approaches have been previously employed by our group to explore the role of anti-malarial agents in special population groups such as paediatrics [50] and pregnant women [51].

The primary objectives of this study were to: (i) assess the impact of dose escalation of ivermectin on adult (20-50 years old) and paediatric (3-5 years old) populations; (ii) assess the impact of inducted based drug-drug interactions on reducing ivermectin plasma concentrations in adults and children and (iii) to assess the impact of optimal dose of ivermectin on a representative malaria population group (Thailand).

The development of ivermectin as a compound file within Simcyp was focussed around utilising existing clinical studies reporting either full plasma concentration-time profiles or sparse sampling time-points with which to develop and drive appropriate predictions of ivermectin concentrations. The studies chosen represented a broad range of single [29,35] and multiple dose studies [29] coupled with standard [36-39] and higher dose studies [29,35].

In the validation of the ivermectin compound file, it was necessary to address the role of active efflux on the intestinal drug absorption, particularly as ivermectin is known to be subjected to active efflux through P-glycoprotein [60]. However, in light of the lack of any *in-vitro* reported kinetic parameters describing active efflux, namely the apparent Vmax (maximum velocity) estimated for the carrier system (Jmax) and the Michaelis constant (km), we incorporated an active efflux component for ivermectin through assuming the active efflux of ivermectin was initially similar to that of digoxin. The impact of this assumption was first confirmed through a sensitivity analysis (Supplementary materials: Section 1), which demonstrated that the choice of digoxin *in vitro* transporter-mediated intrinsic clearance ($CLint_{P\text{-}glycoprotein}$) of 2.5 µL/min, and associated relative activity factor (0.1) was sufficient to capture an appropriate t_{max} and C_{max} for a 60 mg single dose of ivermectin [61].

This approach was further extended to all model simulations in Step 1, and demonstrated successful validation for clinical studies ii-v (see section 2.1) (Figure 2), with all predicted pharmacokinetic parameters residing within the range of literature reported values for all

dosing regimens simulated, and in particular the C_{max}, t_{max} and AUC predictions all within 2-fold of those reported by each clinical study (Table 2).

However, model simulations were unable to capture the AUC_{0-t} reported by Baraka *et al* (1996) [36]. It is possible that the mismatch may have been attributed to the population group utilised for the Baraka study, namely Sudanese, where age-weight relationships have highlighted an overall lower adult weight compared to healthy volunteers (Caucasian) populations [62]. It is also unclear from the Baraka study whether AUC_{0-t} or AUC_{inf} (AUC extrapolated to infinity) was reported. Furthermore, despite this underprediction, our model predicted AUC_{0-t} was within 2-fold of that reported by Okonkwo *et al* (1993) [39], which utilised an identical dose and dosing regimen as Baraka *et al* (1996) [36].

Having successfully demonstrated validation of the ivermectin compound file, we next assessed the impact of dose-escalation on the both the C_{max}, exposure (AUC) and time above the LC50 (16 ng/mL) [31]. Although a key metric for success with antimalarial agents is the day-7 concentration, this information is lacking with ivermectin. The LC50 provides a suitable metric with which to develop an 'exposure-time' relationship. Whilst this has not been fully described within malaria subjects, recent reports have identified LC50 for *Anopheles minimus* (LC50 = 16.3 ng/ml), *Anopheles campestris* (LC50 = 26.4 ng/ml), *Anopheles sawadwongporni* (LC50 = 26.9 ng/ml) and *Anopheles. dirus* (LC50 = 55.6 ng/ml) [31]. Given that *Anopheles minimus* is the primary malaria vector within the GMS [63], it was assumed that an LC50 of 16 ng/mL would form the lower spectrum of a potential therapeutic window. Weight-based dose-escalation over 0.15 mg/kg (standard dose) to 0.60 mg/kg for single doses (Figure 3A) resulted in a clear increase in C_{max} and time above the LC50 (Table 3), with a higher dose of 2 mg/kg (Figure 3B) resulting in a time above the LC50 > 24 hours (Table 3).

A 2 mg/kg dose have been previously clinically administered [29], with the Centre for Disease Control and Prevention recommending doses of up to 1.4 mg/kg for severe crusted scabies [30]. Here, we assumed that a dose of 2 mg/kg would be a realistic 'safe' maximum upper daily dose, given that it was clinically used with no serious adverse reactions in subjects [29]. It was decided to set the upper limit of a possible therapeutic window at the population simulated mean C_{max}, 435.20 ng/mL for adults and 516.91 ng/mL for children.

Therefore, assuming the therapeutic window ranged from 16 ng/mL to 435.20 ng/mL (or 516.91 ng/mL for paediatrics), we assessed the impact of multiple dosing regimens on time

above the LC50 (Figure 3C-F). As expected, a decrease in dosing interval (τ= 24 hours to 12 hours) and increase in dosing regimen duration (3-days or 5 days) resulted in a proportional increase in C_{max} and time above LC50 (Table 3). However, the overall increase in the C_{max} was minimal when comparing single doses with equivalent doses over 3 days (e.g. 0.6 mg/kg single dose: 95.86 ng/mL ± 31.72 ng/mL and daily for 3 days 113.11 ng/mL ± 39.54 ng/mL (Table 3). This was accompanied by an increase in the overall exposure (e.g. 0.6 mg/kg single dose: 960.29 ng/mL.h ± 335.66 ng/mL.h and daily for 3 days 3581.99 ng/mL.h ± 1777.58 ng/mL.h) and associated with an increase in the LC50 from 23.2 hours to 152.9 hours. Thus, the extension of a treatment duration from a single dose to a three-day or five-day treatment regimen would significantly enhance overall ivermectin exposure within the therapeutic window and enhance exposure for approximately 7-11 days. Multiple dosing regimens have previously been used on *Onchocerca volvulus* [64,65] and *Wuchereria bancrofti* [66] and which has been well tolerated.

A key benefit of PBPK modelling is the ability to pragmatically assess the pharmacokinetics of a drug in different population groups, and we next predicted the potential pharmacokinetics in children aged 3-5 years, primarily based upon the recommended weight minimum weight of 15 kg. We attempted to develop both an appropriate therapeutic range in paediatrics and identify the optimal treatment regimens to prolong the time above the LC50. We utilised the same dosing approaches as adults and identified 516.91 ng/mL, as being the potential upper limit for a proposed therapeutic window, based upon doing at 2 mg/kg. Although this is dosing regimen used in adults, it is below the dose of approximately 7-8 mg/kg used in reports of a child who demonstrated ivermectin toxicity [67].

As with adults, increasing single doses (Figure 4A) resulted in increases in C_{max} and AUC with a longer time above the LC50 (Table 4). Furthermore, a similar increase in dosing interval and duration (Figure 4C-E) resulting in a proportional increase in time above the LC50 (Table 4), with the 0.6 mg/kg twice daily for 5 days resulting in the longest time above the LC50 (290.1 ng/mL or 41.4 days), similar to that obtained in adults, 257.19 hours (Table 3).

Thus, for both adults and children, a higher dose of 0.6 mg/kg administered twice daily for 3 or 5 days, leads to significantly higher C_{max} values compared to their corresponding single

daily doses whilst also providing a longer duration above the LC50. When considering the potential problem of the lack of medication compliance with extended dosing of medicines, a 3-day regimen may be an appropriate dosing regimen administered twice daily, to ensure prolonged duration above the LC50 of 9-11 days.

Under standard dosing conditions, a 3-day regimen may be an appropriate way to ensuring prolonged effects. However, many malaria patients are often co-infected with other communicable diseases such as Tuberculosis [41,68-70] or HIV [41]. In these cases, the pharmacotherapy requirements are often complex with multiple competing drug-drug interactions (DDIs) possible. Previously we have illustrated the impact of induction-based DDIs on the reducing the plasma concentration of lumefantrine under dosing with rifampicin (a CYP3A4 inducer) [50], and this step next considered a similar DDI with the use of the antiretroviral efavirenz to simulate HIV-coinfected malaria subjects to ultimately assess the impact of the DDI on reducing ivermectin plasma concentrations.

In all simulations with adults (Figure 5) or paediatrics (Figure 6), the exposure to efavirenz (250 mg once daily for 15 kg to < 20 kg and 300 mg once daily for 20 kg to < 25 kg) significantly reduced ivermectin C_{max}, exposure (AUC) and time above the LC50 for all dosing regimens (Table 5 and 6). The impact of this DDI can be assessed through the AUC ratio or C_{max} ratio, which indicate significant decreases in both AUC ratio (0.21-0.28) and C_{max} ratio (0.39-0.48) for adult studies (Table 5) and a greatest decrease in paediatrics (AUC: 0.19-0.21; C_{max}: 0.36-0.43) (Table 6) across all dosing regimens.

In trying to overcome the reduced exposure of ivermectin in the presence of a CYP3A4 inducer, the use of 1 mg/kg or 2 mg/kg twice daily for five days in adults and children would achieve the greatest time above the LC50 (adults: 138.8 hours and 144.7 hours respectively; paediatrics: 141.2 hours and 142.1 hours respectively).

The focus of this study has generally been towards establishing appropriate dosing regimens for ivermectin for use in malaria infected subjects. However, the marked differences in global age-body weight relationships [62] would clearly alter the establishment of dosing regimens and would, in theory, render a 'one-dose-fits-all' approach inappropriate. Our group has recently utilised a geographic-region specific malaria population group for virtual clinical trials simulation [51]. We adapted this population group for use in the present study and developed a simplistic representative Thailand population group with appropriate body weight distribution for adults and paediatric subjects, whilst also incorporating appropriate

changes in blood biochemistry often observed in malaria-infected patients [51]. Using this approach, we demonstrated a significant difference in the simulated ivermectin plasma concentration from a 0.60 mg/kg daily dose for 3 days regimen (Figure 7A), with a statistically significant 84.7 hours decrease in the time above LC50 in the Thailand population compared to the heathy volunteer population. As dosing was focused on weight-based approaches, the differences in the median body weight for the simulated Thailand population group, 49.86 kg ± 10.25 kg, compared to the healthy volunteer group, 69.41 kg ± 14.29 kg, would therefore alter resultant ivermectin plasma concentration and exemplified the needs to consider population-based age-weight distribution data, as exemplified by the study by Hayes *et al* (2015) [62], to develop more appropriate weight-based dosing regimen for malaria endemic regions. By addressing this potential disparity between body weights, the dosing regimen was adapted to 1 mg/kg, and this could recapture the time above LC50 to a similar extent as that observed in the healthy volunteer population group (Figure 7A). It should be noted that the neutral charge of ivermectin would likely result in preferential binding to human serum albumin (HSA). However, HSA is known to decrease in malaria subjects along with changes in both the haematocrit and alpha-one acidic glycoprotein [51]. This decrease in HSA would be expected to increase both the volume of distribution of ivermectin and more importantly, alter its hepatic extraction, particularly given that ivermectin is highly protein bound [71]. An analysis of the oral clearance demonstrated a significant ($P<0.01$) increase in Thailand subjects compared to healthy volunteers, and this also accounts for the lower overall plasma concentrations. Interesting, a similar trend was not observed in the paediatric population, with simulated ivermectin concentration broadly similar in both population group (Figure 7B)

It should, however, be noted that currently marketed ivermectin contains a mixture termed ivermectin B1a, consisting of an ethyl group at the C-26 position, and ivermectin B1b containing a methyl group [72], in an at least 80% B1a and no more than 20 % B1b mixture [73]. Thus, the possibility of wide variability in ivermectin form within each dosing unit may introduce a wide variation of clinical dose response. Given the possibility of a relatively wide therapeutic window, the impact of such variability may be contained. However further work is required to define the exact duration above the LC50 required to sustain an effect.

The work presented in this study demonstrates the application of PBPK modelling to the successful development and validation of a PBPK model for ivermectin. This has allowed the pragmatic assessment of different dosing regimen designs on ivermectin plasma concentrations *in liue* of clinical trials. Whilst the work presented in this study is not intended to replace future clinical trials assessment of ivermectin in the context of malaria treatment, it can be used to guide and assess other novel dosing regimens or in complex special population groups. However, despite the large number of clinical studies in adults, there is a distinct sparsity in the availability of clinical studies examining ivermectin pharmacokinetics in children, and to fully exploit ivermectin in the context of malaria, urgent clinical trials are required to assess the safety and efficacy of ivermectin in children at doses identified within this study for use in malaria, particularly in the event of an CYP3A4-mediated induction DDI.

Further, the lack of kinetic parameters for P-glycoprotein efflux (Jmax and km) would warrant attention placed on elucidating appropriate *in-vitro* Caco-2 P-glycoprotein kinetic efflux parameters to improve future model predictions. However, using the kinetic parameters associated with digoxin efflux, the model was able appropriately capture this efflux *ab orally* and yield estimates suitable estimates of t_{max} during the model development and for all clinical studies used during the validation stage (Step 1). The model provided will therefore allow for future refinement when this information becomes available.

Given that ivermectin is a highly lipophilic compound [74], it is likely that its oral absorption and oral bioavailability will be enhanced with fat-rich meals, in a similar fashion to other antimalarial agents, e.g. artemether [75,76] and lumefantrine [76]. This would also require consideration of the impact of biorelevant 'fed' dissolution media on the *in-vitro* dissolution rate of ivermectin from a solid dosage formulation. Such data is lacking for the majority of currently used antimalarial agents, and if determined for ivermectin, the proposed model can be adapted to include cumulative percentage release information for fasted and fed states which will allow exploration of the impact of fat-rich meals on ivermectin solubility and dissolution.

CONCLUSION

Although malaria eradication has had wide ranging global successes, the appearance of artemisinin-based combination therapy resistance in the GMS requires urgent attention to the development of new anti-malarial drugs. Traditional discovery/development pipelines may

not accommodate the swift reaction that is required, and repurposing of alternative drug therapies may provide a novel approach to discover new therapies for malaria. Ivermectin is one such drug which has gained attention as a potential candidate. This study has further added to the understanding of the possibility of using ivermectin in a clinical setting within diverse population groups. The dosing regimens simulated are similar to existing therapeutic regimens, and given the wide therapeutic dosing range, provides further support for the repurposing of ivermectin to malaria.

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LIST OF FIGURES

Figure 1: PBPK workflow model

A 6-step workflow model was implemented. Clinical studies utilised for Step 1 ivermectin compound model development and validation are listed in the figure and fully described in Section 2.1.

Figure 2: The simulated plasma concentration-time profile of ivermectin in adults

Ivermectin was dosed based on the reported clinical studies (see Methods section for details) to healthy volunteer adults. Mean observed plasma concentrations are represented by the open circles, with error bars indicating standard deviations on either the reported concentrations (vertical) or reported t_{max} (horizontal). Solid lines represent predicted mean plasma concentration with dashed lines indicating 5th and 95th percentiles. For the study by Na-Bangchang *et al.* (2006), red circles indicate data extracted from complete plasma concentrations profile 'lines' for individual subjects rather than discrete time-points.

Figure 3: The simulated impact of dose escalation on ivermectin plasma concentration-time profiles in healthy volunteer adult subjects

Ivermectin was dosed as: (A) single oral doses of 0.15-0.60 mg/kg; (B) a single 2 mg/kg oral dose; (C) a single daily oral dose of 0.15-0.60 mg/kg for three days; (D) twice daily 0.6 mg/kg oral dose for three days; (E) once daily oral dose of 0.60 mg/kg for five days; (F) twice daily oral doses of 0.60 mg/kg for five days. For all simulations, 100 subjects were simulated with age ranges of 20-50 years. Solid lines represent predicted mean plasma concentrations with dashed lines indicated 5th and 95th percentiles of the lowest and highest doses, where relevant. The dashed horizontal lines indicated the proposed therapeutic window based on the reported LC50 of 16 ng/mL (lower line) and upper (maximum) concentration simulated from the 2-mg single dose study (435.20 ng/mL).

Figure 4: The simulated impact of dose escalation on ivermectin plasma concentration-time profiles in healthy volunteer paediatric subjects

Ivermectin was dosed as (A) single oral doses of 0.15-0.60 mg/kg; (B) a single 2 mg/kg oral dose; (C) single daily oral doses of 0.15-0.60 mg/kg for three days; (D) twice daily oral doses of 0.60 mg/kg for three days; (E) daily oral doses of 0.60 mg/kg for 5 days; (F) twice daily oral doses of 0.60 mg/kg for 5 days. For all simulations 100 subjects were simulated with age ranges of 3-5 years. Solid lines represent predicted mean plasma concentrations with dashed lines indicated 5th and 95th percentiles of the lowest and highest doses, where relevant. The dashed horizontal lines indicated the proposed therapeutic window based on the reported LC50 of 16 ng/mL (lower line) and upper (maximum) concentration simulated from the 2-mg single dose study (516.91 ng/mL).

Figure 5: The simulated impact of an efavirenz-mediated drug-drug interaction on ivermectin plasma concentration-time profiles in healthy volunteer adult subjects

Efavirenz was dosed as single daily 600 mg oral doses throughout the simulation duration with ivermectin dosed on day 13 onwards, under increasing doses from 0.15 mg/kg to 2 mg/kg as: (A) once daily doses; (B) 1 mg/kg and 2 mg/kg as twice daily doses for three days; (C) 1 mg/kg and 2 mg/kg as twice daily doses for five days. For all simulations 100 subjects were simulated with data representing ivermectin plasma concentration profiles in the presence of efavirenz. Solid lines represent predicted mean plasma concentrations with shaded areas indicating 5th and 95th percentiles of the lowest and highest doses respectively. The dashed horizontal lines indicated the proposed therapeutic window based on the reported LC50 of 16 ng/mL (lower line) and upper (maximum) concentration simulated from the 2-mg single dose study (435.20 ng/mL).

Figure 6: The simulated impact of an efavirenz-mediated drug-drug interaction on ivermectin plasma concentration-time profiles in healthy volunteer paediatric subjects

Efavirenz was dosed as single daily 250 mg (15-20 kg) or 300 mg (20-25 kg) oral doses throughout the simulation duration with ivermectin dosed on day 13 onwards under increasing doses from 0.60 mg/kg to 2 mg/kg as: (A) once daily doses; (B) 1 mg/kg and 2 mg/kg as twice daily doses for three days; (C) 1 mg/kg and 2 mg/kg as twice daily doses for

five days. For all simulations, 100 subjects were simulated with data representing ivermectin plasma concentration profiles in the presence of efavirenz. Data for both the 250mg and 300 mg efavirenz dose were pooled, and the mean presented, with simulations containing at least 50 subjects within each dosing band. Solid lines represent predicted mean plasma concentrations with shaded regions indicating 5th and 95th percentiles of the lowest and highest doses respectively. The dashed horizontal lines indicated the proposed therapeutic window based on the reported LC50 of 16 ng/mL (lower line) and upper (maximum) concentration simulated from the 2-mg single dose study (516.91 ng/mL).

Figure 7: Simulated ivermectin plasma concentration in adult and paediatric malaria population group

Ivermectin was dosed at 0.60 mg/kg or 1 mg/kg to adults (20-50 years) and paediatrics (3-5 years) under 3-day dosing (black and red) or 5-day dosing (green). The healthy volunteer population group (Caucasian) was used as a default population group with the Thailand population group created with appropriate age-weight distributions and changes in blood biochemistry to mimic a malaria population group. For all simulations, 100 subjects were simulated. Solid lines represent predicted mean plasma concentrations with shaded regions indicating 5th and 95th percentiles of the Thailand malaria and Caucasian populations, respectively. The dashed horizontal lines indicated the proposed therapeutic window based on the reported LC50 of 16 ng/mL (lower line) and upper (maximum) concentration simulated from the 2-mg single dose study.

LIST OF TABLES

Table 1. Final optimised ivermectin parameters for multi-dose simulations

Parameters	Value	Notes
Compound type	Neutral	
Molecular weight (g/mol)	875.1 [1]	
Log P	5.8 [2]	
fu	0.068 [3]	
pKa 1	-	
pKa 2	-	
B/P	3.62	Predicted by Simcyp Prediction Toolbox
Vss (L/kg)	1.34	Final optimised using a
SAC (L/kg)	0.179	minimal PBPK model
k_{in} (h^{-1})	0.1751	with a SAC [a]
k_{out} (h^{-1})	0.0336	
Papp ($x10^{-6}$ cm/s)	7.6 [4]	
$CLint_{P\text{-}glycoprotein}$ (µL/min)	2.5 [b]	
RAF	0.1 [b]	
k_a (h^{-1})	0.38	Estimated from Peff
fa	0.69	Estimated from Peff
CL_{po} (L/h)	21.25 [c]	Mean from literature
$CLint_{3A4}$ (µL/min/pmol)	0.28 [c]	Final optimised
Absorption model	ADAM	
Distribution model	Minimal	

[a] Parameter estimated using a minimal PBPK model with a single adjusting compartment (SAC). [b] The contribution of active efflux to ivermectin intestinal absorption was assumed to be similar to that of the reported value for digoxin [5], with RAF empirically optimised through a sensitivity analysis (see supplementary materials). [c] $CLint_{CYP}$ was based on a retrograde calculation, described in Step 1, with fa fixed at 0.56 and F_G assumed = 1. Final estimates were obtained through parameter estimation assuming an fmcyp of 1 for CYP3A4.

Log P: octanol/water partition coefficient; fu: unbound fraction; B/P: blood-to-plasma ratio; Vss: steady state volume of distribution; k_a: absorption rate constant; fa: fraction dose absorbed; CL_{po}: oral clearance; CLint: *in vitro* intrinsic clearance for active efflux (P-glycoprotein) or metabolism (3A4); F_G: fraction of drug escaping the gut enterocyte intact; RAF: relative activity factor.

Table 2: Summary of predicted and observed pharmacokinetic parameters of ivermectin used in the validation

Study Name and Dose		C_{max} (ng/mL)		t_{max} (h)		$AUC_{0\text{-}inf}$ or $AUC_{0\text{-}time}$ (ng/mL.h)	
		Predicted	*Observed*	*Predicted*	*Observed*	*Predicted*	*Observed*
Guzzo: 30mg [6]	*Day 1*	77.82 ± 31.12	84.8 ± 42.7	3.52 ± 0.4	4.3 ± 1	1629.23 ± 650.58	1724.3 ± 830.5
	Day 7	99.85 ± 58.25	87.0 ± 42.2	3.79 ± 1.2	4.2 ± 0.9	3239.82 ± 1356.66	2819.4 ± 1691.2
Guzzo: 60mg [6]	*Day 1*	114.23 ± 102.99	165.2 ± 95.6	3.11 ± 0.8	3.6 ± 0.9	2429.63 ± 1311.74	2984 ± 1530.1
	Day 7	162.87 ± 143.13	186.2 ± 130.8	3.40 ± 1.2	4.0 ± 1.1	6187.15 ± 3982.52	6061.7 ± 4243.7
Guzzo: 90 mg [6]	*Single*	151.63 ± 95.26	158.1 ± 87.6	4.11 ± 0.85	4.9 ± 1.8	3814.24 ± 1324.07	2910.2 ± 1801.9
Guzzo: 120 mg [6]	*Single*	171.33 ± 112.28	247.8 ± 158.9	4.18 ± 0.89	4.2 ± 0.9	5124.61 ± 1498.92	4547.7 ± 2402.9
Edwards: 12 mg [7]	*Single*	40.29 ± 13.36	46 ± 20	3.40 ± 0.35	3.6 ± 0.7	588.71 ± 211.19	885 ± 389
Baraka: 0.15 µg/kg [8]	*Single*	49.62 ± 11.36	54.4 ± 12.2	3.40 ± 0.31	4.9 ± 1.5	797.31 ± 157.33	3180 ± 1390
Na-Bangchang: 0.2 µg/kg [9]	*Single*	54.01 ± 14.51	-	3.70 ± 0.3	-	1609.22 ± 578.24	-
Njoo: 0.15 µg/mL [10]	*Single*	39.94 ± 9.31	-	3.67 ± 0.29	-	1229.27 ± 436.68	-
Okonkwo: 0.15 µg/mL [11]	*Single*	40.45 ± 15.62	38.2 ± 16.15	3.73 ± 0.58	4.7 ± 1.49	862.12 ± 277.27	1545.3 ± 537.4

Data represent mean ± SD; $AUC_{0\text{-}time}$ calculated for studies by Okonkwo [11] and Edwards [7].

$AUC_{0\text{-}time}$: AUC calculated for the study period only; $AUC_{0\text{-}inf}$: AUC calculated from the start of the study and extrapolated to infinity.

Table 3: Simulated pharmacokinetic parameters of ivermectin under dose escalation in healthy adult subjects

Duration	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{final\ dose\text{-}t}$ (ng/mL.h)	Time above LC50 (h)
Single	0.15	35.47 ± 8.77	3.41 ± 0.36	342.58 ± 89.93	10.4
	0.3	60.52 ± 15.12	3.49 ± 0.39	595.75 ± 164.26	15.8
	0.6	95.86 ± 31.72	3.55 ± 0.40	960.29 ± 335.66	23.2
	2	178.38 ± 95.98	3.70 ± 0.42	1779.92 ± 890.56	> 24
3 Days	0.15	41.11 ± 10.48	3.40 ± 0.37	1255.36 ± 522.29	32.2
	0.3	70.80 ± 20.03	3.47 ± 0.38	2213.06 ± 1003.6	86.2
	0.6	113.11 ± 39.54	3.49 ± 0.37	3581.99 ± 1777.58	152.9
	0.6 BD	151.51 ± 66.22	3.29 ± 0.34	6292.28 ± 3659.18	178.2
5 Days	0.6	124.54 ± 53.19	3.51 ± 0.37	4543.99 ± 2513.48	182.3
	0.6 BD	174.41 ± 73.69	3.30 ± 0.35	8024.87 ± 4667.20	257.1

Data represents median ± SD. n=100. For 3- and 5-day simulations, AUC was calculated from the final dosing period to the end of the study period. Time above LC50 (16 ng/mL) was calculated from the median line of each simulation. BD: twice daily.

Table 4: Simulated pharmacokinetic parameters of ivermectin under dose escalation in healthy paediatric subjects

Duration	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{final\ dose\text{-}t}$ (ng/mL.h)	Time above LC50 (h)
Single	0.15	42.92 ± 8.91	3.60 ± 0.46	394.10 ± 87.71	10.1
	0.3	81.41 ± 19.85	3.67 ± 0.56	763.97 ± 202.29	14.6
	0.6	145.07 ± 41.43	3.75 ± 0.59	1397.10 ± 444.39	23.9
	2	348.40 ± 148.95	3.98 ± 0.75	3423.15 ± 1506.71	> 24
3 Days	0.15	51.02 ± 10.31	3.58 ± 0.45	1454.62 ± 600.10	37.1
	0.3	97.39 ± 23.83	3.64 ± 0.52	2858.04 ± 1252.65	88.1
	0.6	174.85 ± 51.30	3.72 ± 0.58	5340.55 ± 2593.37	151.2
	0.6 BD	225.54 ± 80.71	3.56 ± 0.54	9109.37 ± 4790.91	214.5
5 Days	0.6	206.22 ± 62.35	3.58 ± 0.51	7278.17 ± 3843.94	234.5
	0.6 BD	263.82 ± 98.72	3.48 ± 0.48	11712.94 ± 6438.28	290.1

Data represents median ± SD. *n*=100. For 3- and 5-day simulations, AUC was calculated from the final dosing period to the end of the study period. Time above LC50 (16 ng/mL) was calculated from the median line of each simulation. BD: twice daily.

Table 5: Simulated pharmacokinetic parameters of ivermectin in the presence of an efavirenz-mediated drug-drug interactions in healthy adult subjects

Duration	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{final\ dose\text{-}t}$ (ng/mL.h)	AUC Ratio	Cmax Ratio	Time above LC50 (h)
3 Days	0.15	23.61 ± 7.92	2.77 ± 0.38	264.23 ± 115.69	0.28 ± 0.06	0.48 ± 0.10	9.3
	0.3	40.78 ± 14.53	2.78 ± 0.38	462.28 ± 203.15	0.28 ± 0.06	0.48 ± 0.10	19.7
	0.6	65.40 ± 25.89	2.83 ± 0.39	740.13 ± 325.21	0.28 ± 0.06	0.48 ± 0.10	24.2
	1	82.12 ± 38.98	2.87 ± 0.37	1052.84 ± 476.63	0.28 ± 0.06	0.48 ± 0.10	30.8
	2	120.39 ± 61.70	2.92 ± 0.37	1360.83 ± 645.68	0.28 ± 0.06	0.48 ± 0.10	47.5
	1 BD	99.72 ± 48.73	2.78 ± 0.22	1533.18 ± 810.62	0.23 ± 0.06	0.42 ± 0.08	77.3
	2 BD	136.16 ± 72.64	2.89 ± 0.36	1879.75 ± 973.82	0.25 ± 0.06	0.42 ± 0.08	91.2
5 Days	1 BD	106.4 ± 51.37	2.80 ± 0.31	1623.55 ± 865.22	0.23 ± 0.06	0.40 ± 0.07	138.8
	2 BD	145.67 ± 76.98	2.84 ± 0.32	2347.87 ± 1217.91	0.21 ± 0.06	0.39 ± 0.07	144.7

Data represent median ± SD in the presence of efavirenz. n=100.

For 3- and 5-day simulations, $AUC_{final\ dose\text{-}t}$ was calculated from the final dosing period to the end of the study period. Time above LC50 (16 ng/mL) was calculated from the median line of each simulation. BD: twice daily.

Table 6: Simulated pharmacokinetic parameters of ivermectin in the presence of an efavirenz-mediated drug-drug interaction in healthy paediatric subjects

Duration	Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{final\ dose\text{-}t}$ (ng/mL.h)	AUC Ratio	C_{max} Ratio	Time above LC50 (h)
3 Days	0.6	98.25 ± 55.72	2.88 ± 0.47	909.93 ± 579.05	0.21 ± 0.10	0.43 ± 0.13	27.8
	1	159.28 ± 92.85	3.00 ± 0.56	1799.69 ± 987.56	0.21 ± 0.09	0.43 ± 0.12	30.9
	2	240.45 ± 150.97	3.08 ± 0.59	2225.53 ± 1503.45	0.21 ± 0.09	0.43 ± 0.12	30.9
	1 BD	155.06 ± 90.31	2.98 ± 0.55	1754.32 ± 1144.67	0.19 ± 0.09	0.37 ± 0.12	81.2
	2 BD	257.42 ± 162.43	3.07 ± 0.64	2812.20 ± 1929.57	0.19 ± 0.09	0.38 ± 0.12	104.2
5 Days	1 BD	176.32 ± 98.71	3.06 ± 0.46	2071.25 ± 1347.96	0.20 ± 0.09	0.36 ± 0.12	141.2
	2 BD	274.51 ± 165.31	3.11 ± 0.58	3114.23 ± 2006.82	0.20 ± 0.09	0.36 ± 0.12	142.1

Data represent median ± SD in the presence of efavirenz. n=100.

For 3- and 5-day simulations, $AUC_{final\ dose\text{-}t}$ was calculated from the final dosing period to the end of the study period. Time above LC50 (16 ng/mL) was calculated from the median line of each simulation. BD: twice daily.

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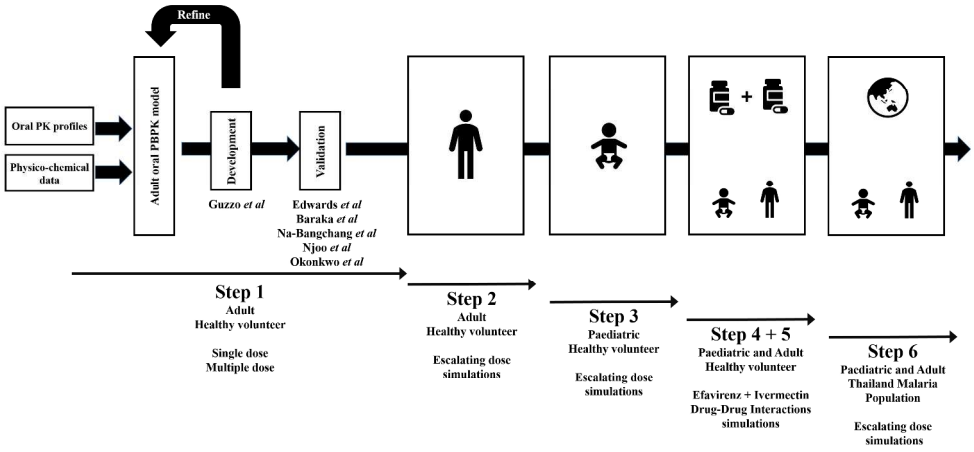

Figure 1

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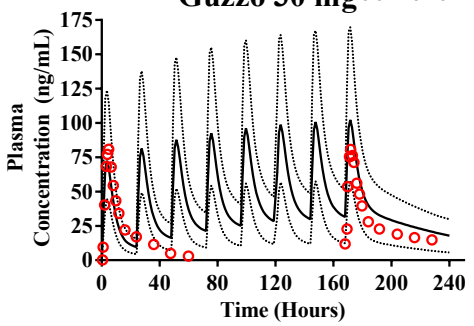
Guzzo 30 mg
Plasma Concentration (ng/mL)
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150
125
100
75
50
25
0
0
40
80
120
160
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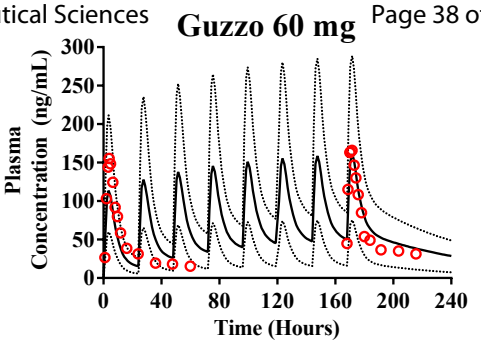
240
Time (Hours)


Guzzo 60 mg
Plasma Concentration (ng/mL)
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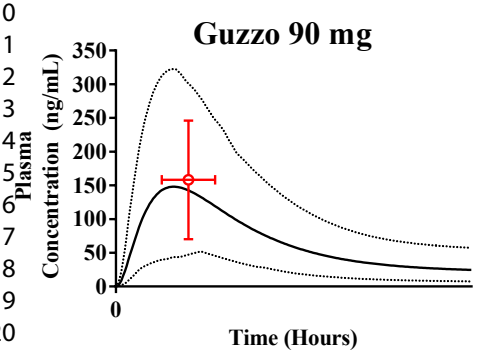
200
150
100
50
0
0
40
80
120
160
200
240
Time (Hours)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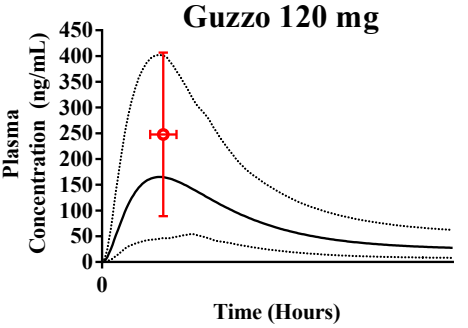
300
250
200
150
100
50
0
0
Time (Hours)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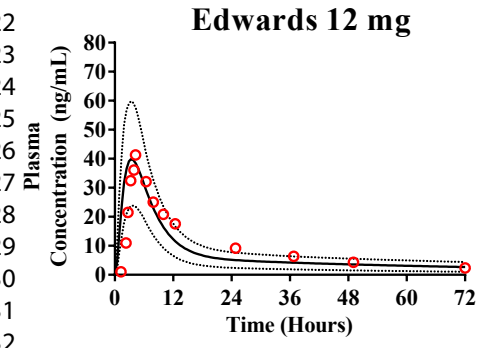
300
250
200
150
100
50
0
0
Time (Hours)


Edwards 12 mg
Plasma Concentration (ng/mL)
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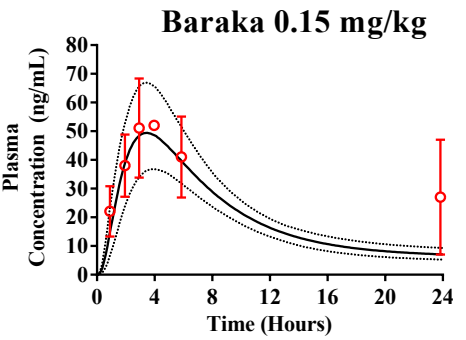
60
50
40
30
20
10
0
0
12
24
36
48
60
72
Time (Hours)


Baraka 0.15 mg/kg
Plasma Concentration (ng/mL)
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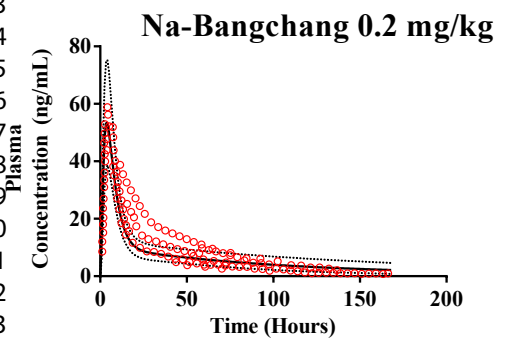
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
Time (Hours)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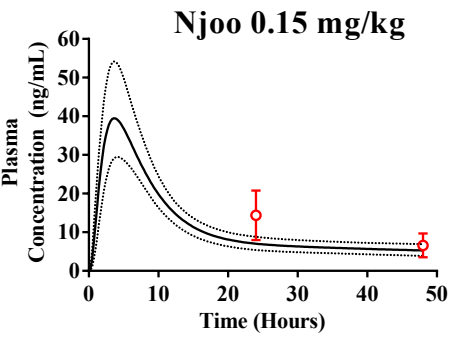
60
40
20
0
0
50
100
150
200
Time (Hours)


Njoo 0.15 mg/kg
Plasma Concentration (ng/mL)
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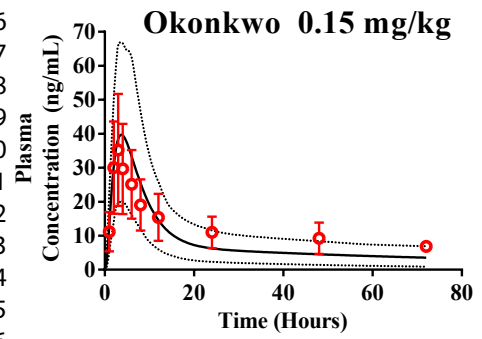
50
40
30
20
10
0
0
10
20
30
40
50
Time (Hours)


Okonkwo 0.15 mg/kg
Plasma Concentration (ng/mL)
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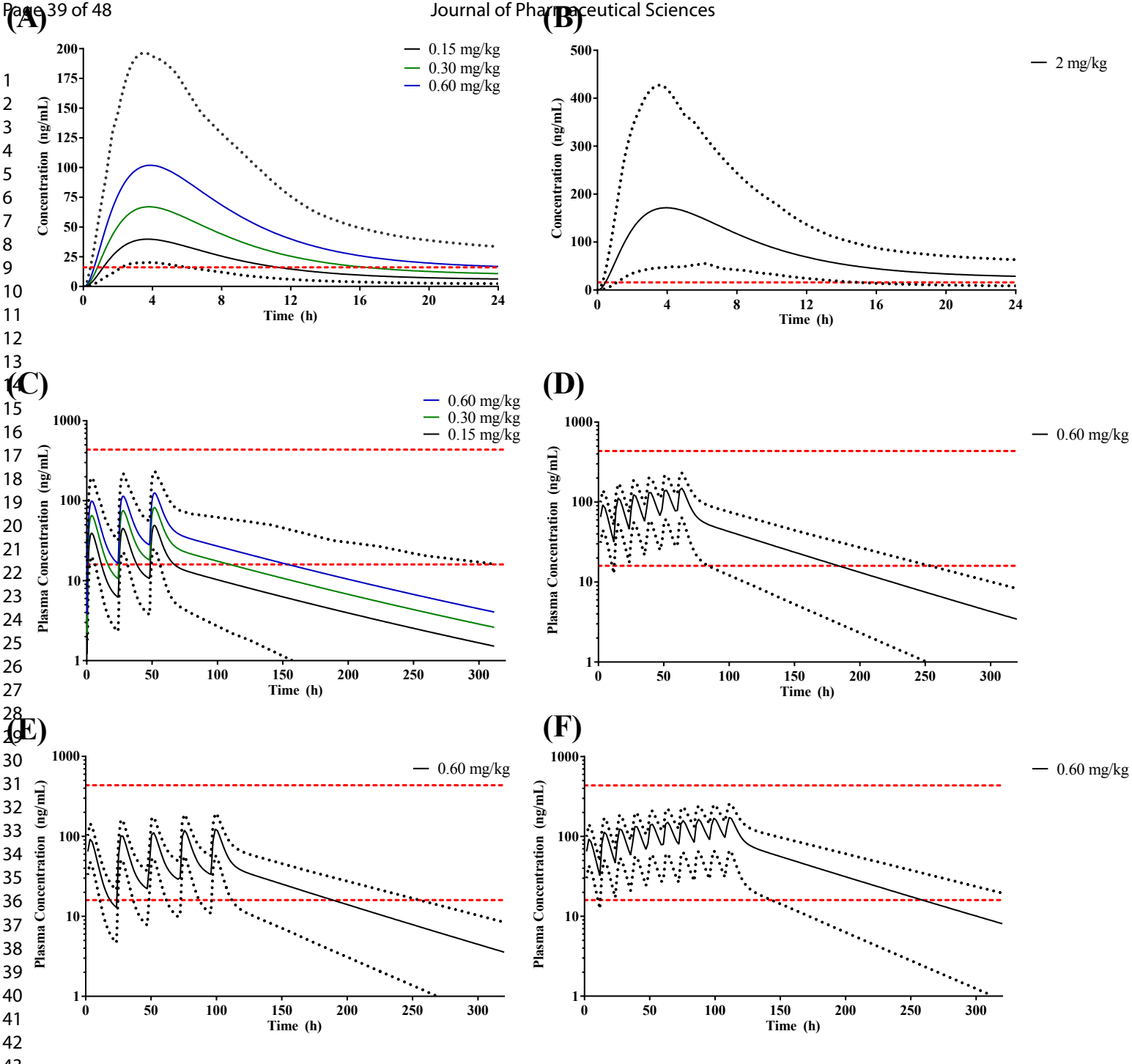
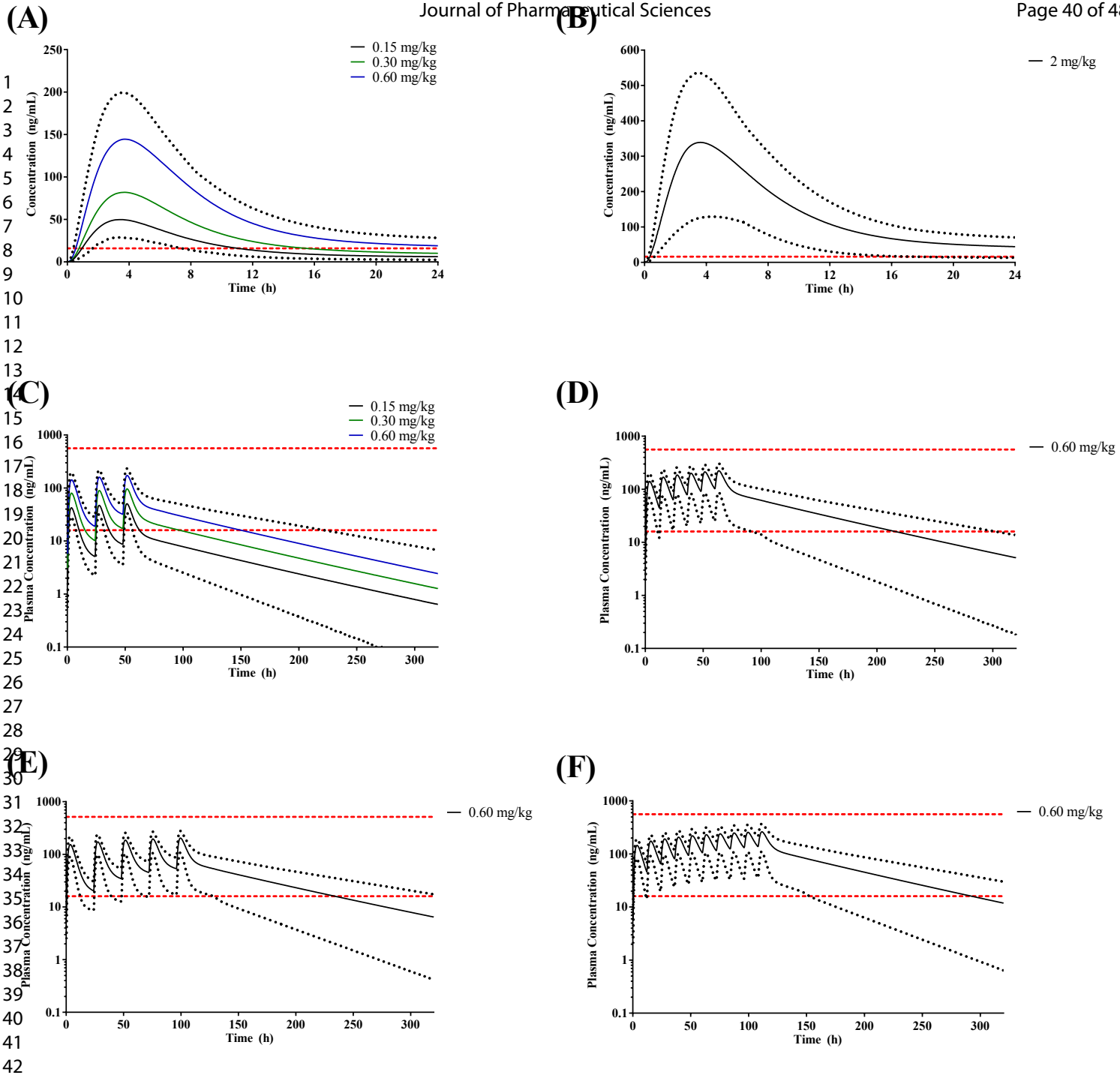
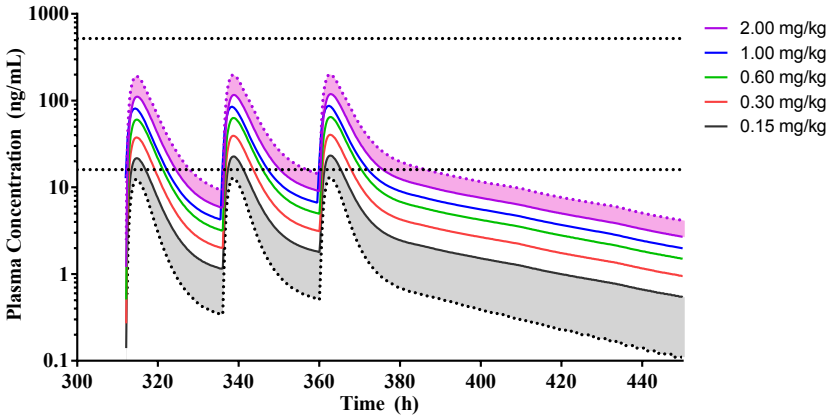
60
50
40
30
20
10
0
0
20
40
60
80
Time (Hours)

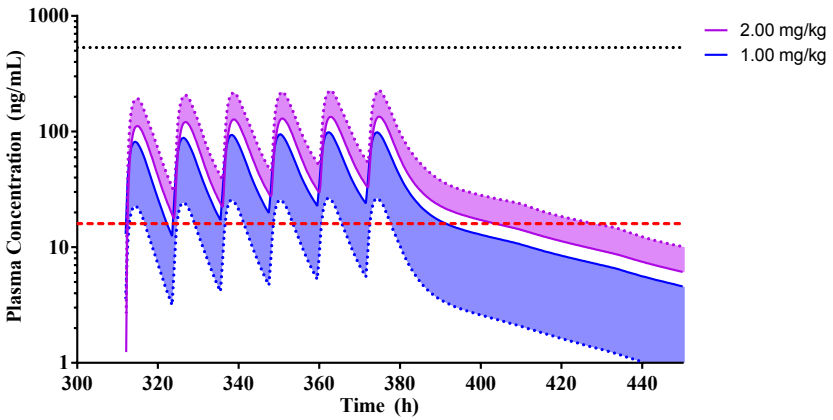

(A)
0.15 mg/kg
0.30 mg/kg
0.60 mg/kg
Concentration (ng/mL)
Time (h)
(B)
2 mg/kg
Concentration (ng/mL)
Time (h)
(C)
0.60 mg/kg
0.30 mg/kg
0.15 mg/kg
Plasma Concentration (ng/mL)
Time (h)
(D)
0.60 mg/kg
Plasma Concentration (ng/mL)
Time (h)
(E)
0.60 mg/kg
Plasma Concentration (ng/mL)
Time (h)
(F)
0.60 mg/kg
Plasma Concentration (ng/mL)
Time (h)


(A)
0.15 mg/kg
0.30 mg/kg
0.60 mg/kg
Concentration (ng/mL)
Time (h)
(B)
2 mg/kg
Concentration (ng/mL)
Time (h)
(C)
0.15 mg/kg
0.30 mg/kg
0.60 mg/kg
Plasma Concentration (ng/mL)
Time (h)
(D)
0.60 mg/kg
Plasma Concentration (ng/mL)
Time (h)
(E)
0.60 mg/kg
Plasma Concentration (ng/mL)
Time (h)
(F)
0.60 mg/kg
Plasma Concentration (ng/mL)
Time (h)

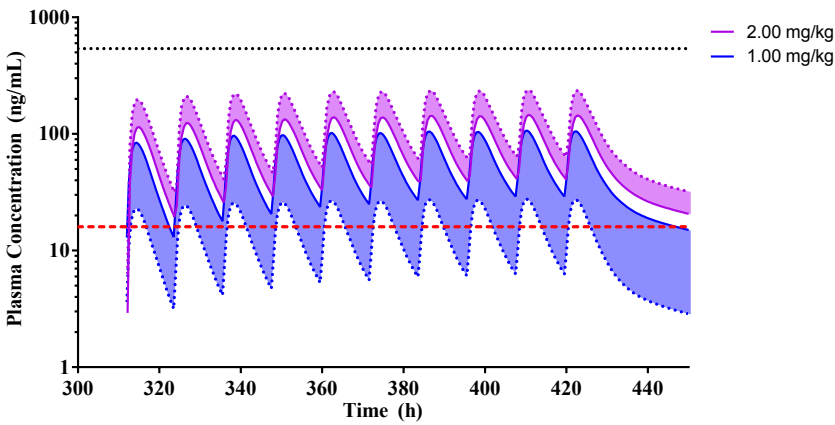
(A)

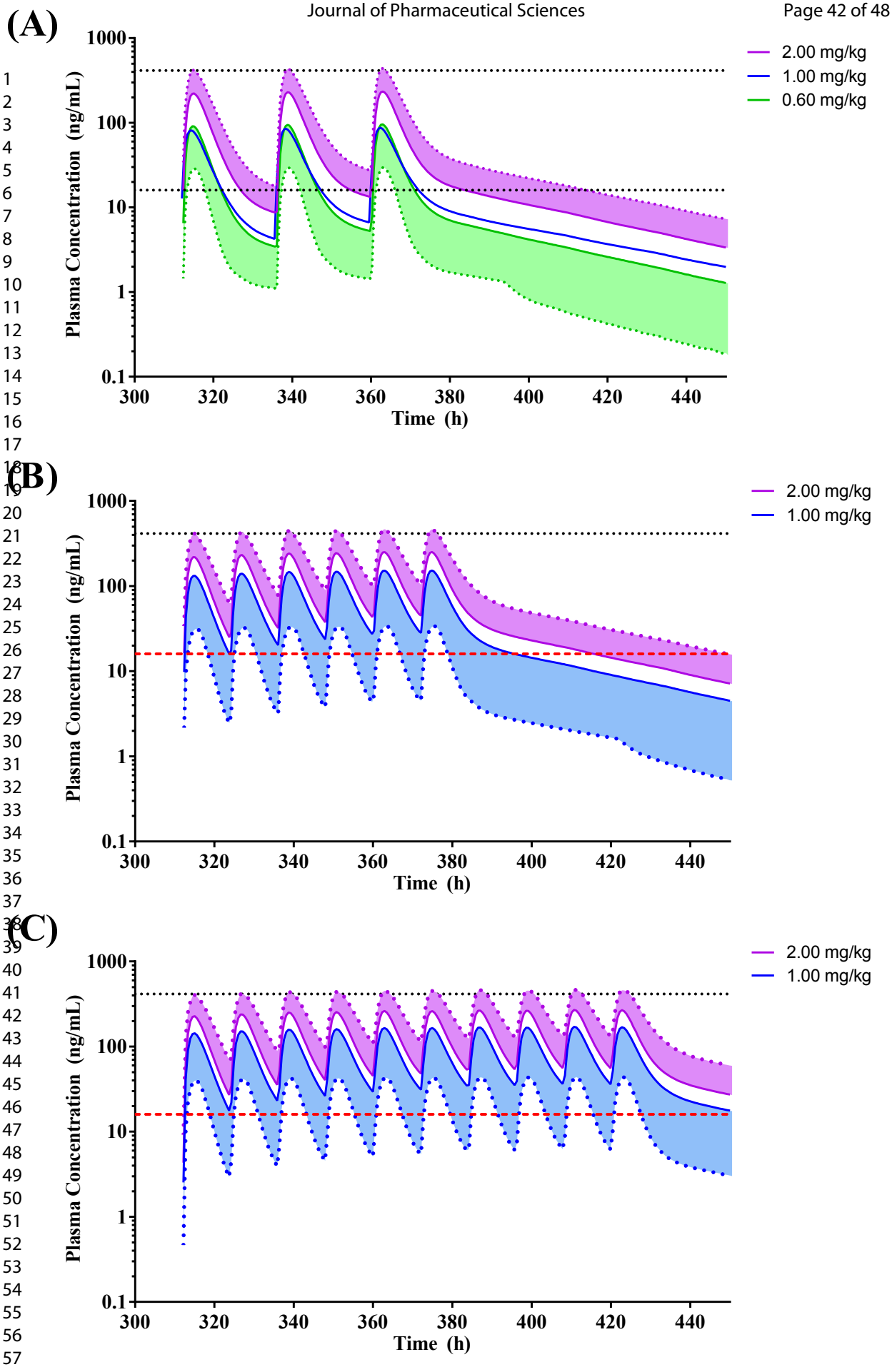

Plasma Concentration (ng/mL)
1000
100
10
1
0.1
300
320
340
360
380
400
420
440
Time (h)
2.00 mg/kg
1.00 mg/kg
0.60 mg/kg
0.30 mg/kg
0.15 mg/kg

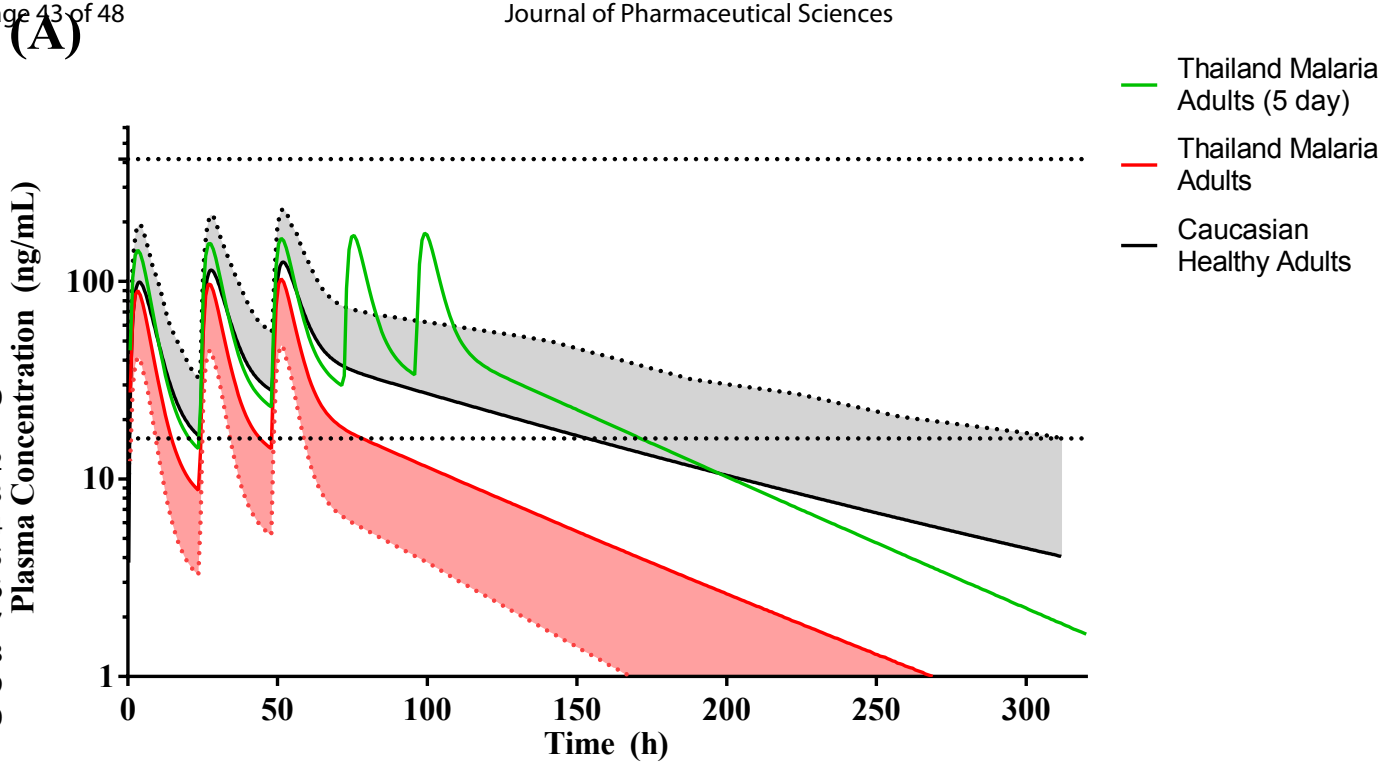
(B)

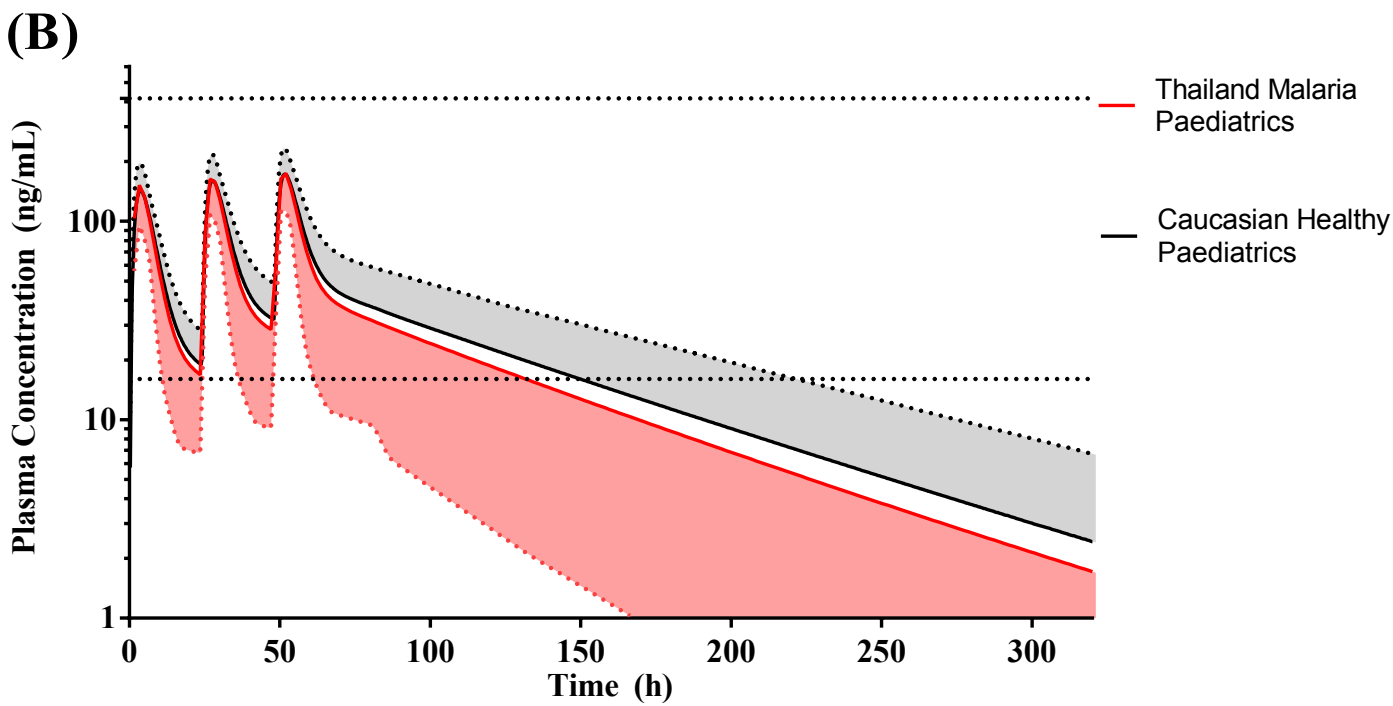

Plasma Concentration (ng/mL)
1000
100
10
1
300
320
340
360
380
400
420
440
Time (h)
2.00 mg/kg
1.00 mg/kg

(C)


Plasma Concentration (ng/mL)
1000
100
10
1
300
320
340
360
380
400
420
440
Time (h)
2.00 mg/kg
1.00 mg/kg


(A)
2.00 mg/kg
1.00 mg/kg
0.60 mg/kg
Plasma Concentration (ng/mL)
1000
100
10
1
0.1
300
320
340
360
380
400
420
440
Time (h)
(B)
2.00 mg/kg
1.00 mg/kg
Plasma Concentration (ng/mL)
1000
100
10
1
0.1
300
320
340
360
380
400
420
440
Time (h)
(C)
2.00 mg/kg
1.00 mg/kg
Plasma Concentration (ng/mL)
1000
100
10
1
0.1
300
320
340
360
380
400
420
440
Time (h)


(A)
Thailand Malaria Adults (5 day)
Thailand Malaria Adults
Caucasian Healthy Adults
Plasma Concentration (ng/mL)
100
10
1
0
50
100
150
200
250
300
Time (h)


(B)
Thailand Malaria Paediatrics
Caucasian Healthy Paediatrics
Plasma Concentration (ng/mL)
100
10
1
0
50
100
150
200
250
300
Time (h)

Supplementary materials

Section 1: Model development

Steady-state volume of distribution (Vss)

To recover the shape of the distribution and elimination phases of the plasma-concentration time profiles, estimation of the steady-state volume of distribution (Vss) was determined from published clinical data through parameter estimation with observed plasma concentration-time profiles using a weighted least square algorithm with a Nelder-Mead minimisation method, to yield a Vss of 1.343 L/kg using a minimal PBPK model. Estimation of the single adjustment compartments (SAC) was 0.179 L/kg with inter-compartmental transfer constants k_{in} and k_{out} of 0.1751 h^{-1} and 0.0336 h^{-1}.

Metabolic Intrinsic clearance (CLint)

The ready availability of *in-vitro* metabolic intrinsic clearance data is limited for ivermectin. However, it has been identified that CYP3A4 is the major metabolic pathway [1]. It was therefore assumed that the major pathway would be attributed to CYP3A4 with an intrinsic clearance ($CLint_{3A4}$) estimated using the Simcyp retrograde calculator using a fixed CL_{oral} of 21.25 L/h, the mean of 5 reported individual CL_{oral} [2,3] (and assuming fa~0.56 [4]), with CYP3A4 allocated 100 % of the total clearance. The final predicted $CLint_{3A4}$ was 0.28 µL/min/pmol. Renal clearance has been reported to be negligible [5] and therefore was not considered within the model.

Passive permeability

Ivermectin is a low solubility BCS Class II compound, and therefore permeability is thought to be limited. As a result of the lack of a range of published *in-vitro* Caco-2 permeability measurements, a single published study was utilised which reported an *in-vitro* apparent permeability ($Papp_{AB}$) of $7.6x10^{-6}$ cm/s [6]. This was then used in the Simcyp ADAM model to estimate a human jejunum effective permeability (Peff) of $0.88x10^{-4}$ cm/s. Subsequently, this was then used to estimate the absorption rate constant (k_a) and fraction dose absorbed (fa) using the ADAM model resulting in an initial estimate of 0.38 h^{-1} and 0.69 for k_a and fa respectively. However, attempts to capture an appropriate C_{max} and t_{max} for ivermectin (~ 4-6 hours) [2,7,8] failed. As ivermectin has also been reported to be a P-glycoprotein substrate [6,9],

the contribution of active efflux on limiting intestinal absorption and hence delaying t_{max} was modelled by the inclusion of an active efflux component into the model.

Active efflux

Recently Zhou *et al* (2016) [10] reported the successful development of a Simcyp model for naloxegol. In the absence of *in-vitro* reported kinetic efflux parameters, they utilised the Simcyp default digoxin efflux kinetic parameters as a surrogate for the active efflux of naloxegol. This approach resulted in the successful development of a PBPK model for naloxegol.

As ivermectin P-glycoprotein-specific Michaelis-Menten efflux kinetic parameters are absent in the literature, namely the apparent Vmax (maximum velocity) estimated for the carrier system (Jmax) and the Michaelis constant (km), assumptions were made to obtain a reasonable absorption phase profile of ivermectin. We therefore utilised a similar approach as that implemented by Zhou et al (2016) [10], where the default *in-vitro* transporter-mediated intrinsic clearance ($CLint_{P\text{-}glycoprotein}$) value for digoxin (2.5 µL/min) [11] along with the default Simcyp validated Relative Activity Factor (RAF) (enables *in-vitro* to *in-vivo* scaling of transport clearances) were used as a surrogate for ivermectin efflux.

Subsequently we conducted a sensitivity analysis to assess this assumption through exploring the impact of changes in $CLint_{P\text{-}glycoprotein}$ (1-12 µL/min) and RAF (0-1) on ivermectin C_{max} and t_{max} (Figure 1), where a 60 mg single oral dose was administered to healthy subjects to mimic the study reported by Guzzo *et al.* (2002) [12]. The impact of increasing $CLint_{P\text{-}glycoprotein}$ on ivermectin C_{max} is significant when $CLint_{P\text{-}glycoprotein}$ and RAF both increase (Figure 1A), with an equally significant increase in the simulated t_{max} (Figure 1B). An empirical assessment of the sensitivity analysis identified an ivermectin RAF of 0.1 would enable a more appropriate estimate of both the ivermectin C_{max} and t_{max} when compared to Guzzo *et al.* (2002) [12]. When this revised RAF was incorporated into simulations, the model was adequately able to capture the reported C_{max} and t_{max} for the 60 mg single dose, namely 165.2 ng/ml ± 95.6 ng/ml and 3.6 h ± 0.9 h.

Further, to ensure these parameter values were appropriate for lower doses, these parameters were also used in validation steps using clinical studies ii-v (See Methods Step 1) at body weight based doses of 0.15 mg/kg-0.2 mg/kg (~10-12 mg assuming and average body weight of 75 kg) and single doses of 12 mg.

The finalised kinetic parameters describing ivermectin efflux were incorporated into the compound file as an $CLint_{P\text{-}glycoprotein}$ of 2.5 µL/min and a RAF of 0.1.

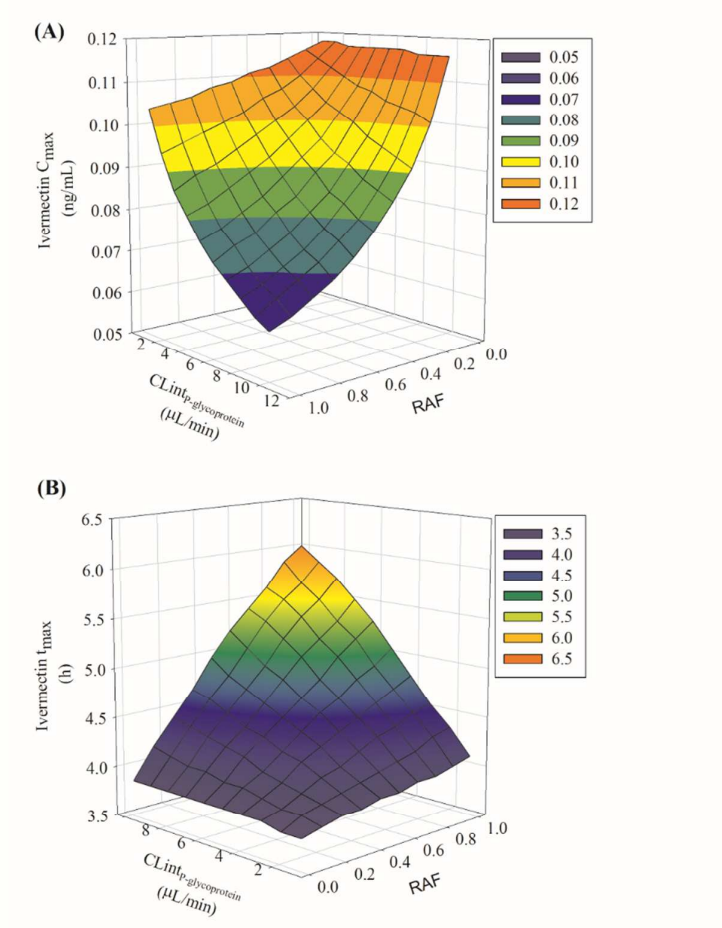


Figure 1: Sensitivity analysis of active efflux and efflux scaling factor on ivermectin C_{max} and t_{max}.

The sensitivity of P-glycoprotein active efflux clearance ($CLint_{P\text{-}glycoprotein}$) and relative activity factor (RAF) on simulated ivermectin C_{max} (A) or t_{max} (B). A 60 mg oral dose was administered to a single healthy subject and the sensitivity of $CLint_{P\text{-}glycoprotein}$ (1-10 µL/min) and RAF (0.10-1) C_{max} (A) or t_{max} (B) simulated over 100 simulations.

Solubility

All dosing was conducted using a solid immediate release dosage form, with dissolution controlled by the intrinsic aqueous solubility with a Simcyp estimate of 0.0013 mg/mL

(estimate in literature: < 0.005 mg/mL [13]) assuming a melting point of 155 °C and using an empirical predictor equation developed by Jain and Yalkowsky [14].

Section 2: Thailand population group

The age-weight distribution for male and female Thailand adult and paediatric subjects were extracted from age-weight distribution profiles developed by Hayes *et al* (2015) [15] and polynomial/linear equations applied to describe the shape of profiles using an approach described and implemented previously by our group [16].

The resultant mathematical expression of age-weight distribution are detailed below:

Adult Males

$$\text{Weight} = 33.46 + (-0.3569*\text{age}^2) + (0.001522*\text{age}^4) / (1 + (-0.00755*\text{age}^2) + (2.78\text{x}10^{-5}*\text{age}^4) + (-1.07\text{x}10^{-9}*\text{age}^6))$$

Paediatric Males

$$\text{Weight} = 5.0164 + (1.74*\text{age})$$

Adult Females

$$\text{Weight} = -920.66 + (-188.63*\text{age}) + (22.48*\text{age}^{1.5}) + (-0.999*\text{age}^2) + (700.23*\text{age}^{0.5})$$

Paediatric Females

$$\text{Weight} = (5.635 + 1.121*\text{age}) / (1 + -0.0282*\text{age})$$

For paediatric population groups, the age-weight relationship was calculated from 2-6 years of age. In the absence of appropriate age-height distributions, the relationship was assumed to be similar to that described by Simcyp for a healthy volunteer population group.

Blood biochemistry alterations (haematocrit and serum proteins) were also incorporated into the Thailand population group as described previously by our group [16].

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