SUPPORTING INFORMATION

Study design

Patients aged 2 to 10 years who presented at the public health facilities with symptoms suggestive of malaria (fever or history of fever) were referred to the outpatient laboratory for finger prick screening using Giemsa-stained thick blood smears. At each visit and on any day of parasite recurrence, 3-4 drops of blood were saved onto filter paper (Watmann 3MM) for genotyping purposes. Parasite density was estimated by counting the number of asexual parasites per 200 leukocytes, assuming a leukocyte count of 8,000/µl. All patients with a *P. falciparum* mono-infection parasite density of 2,000-200,000/uL were referred to the study clinician for further evaluation. Enrolment criteria were: older than 6 months, body weight above 5 kg, axillary fever above 37.5 ºC or history of fever in the previous 24 hours, no history of serious side effects to study medications, no evidence of a concomitant febrile illness in addition to malaria, no history of antimalarial use in the previous two weeks, and no signs or evidence of severe malaria (unrousable coma, recent febrile convulsions, altered consciousness, lethargy, unable to drink or breast feed, severe vomiting, unable to stand/sit due to weakness, severe anaemia (Hb<5.0 gm/dL), respiratory distress, and/or jaundice). If consent was forthcoming, a full medical history and examination were carried out by the physician and the results were recorded. All patients who did not satisfy the selection criteria for participating in the study were referred back to the outpatient department for appropriate care.

Blood samples

A baseline blood sample was taken before any treatment was given. Capillary blood samples (approximately 200 µL) were taken from a finger prick and placed in heparinised tubes on days 2, 3, 4, 5, 7, 14, 21, 28, 35, and 42 after initial drug administration. Capillary samples were chosen to minimize the invasiveness of the study in small children and to ensure a high level of compliance to the study protocol. An additional venous blood sample (2-5 mL) was drawn by venipuncture from all patients at day 7 to correlate venous and capillary concentrations. The exact time of each sampling was recorded and used in the pharmacokinetic analysis. Capillary and venous blood samples were centrifuged at 2,000 g at room temperature for 10 min, and plasma was then placed immediately in screw cap cryo-tubes and stored at -20°C. Blood samples were subsequently transported on dry ice to the Department of Clinical Pharmacology, Faculty of Tropical Medicine, Mahidol University in Bangkok, Thailand.

Pharmacokinetic analysis

Estimation and simulation of concentration-time data were carried out using a nonlinear mixed-effects approach in the NONMEM software version 6 (ICON Development Solutions, MD, USA). Automation and post-processing was performed using Pearl-Speaks-NONMEM (PsN) version 3.2.4 (33-34), Census version 1.1 (35) and Xpose version 4.0 (36). The first-order conditional estimation method with interactions was used throughout the modelling (37). Model selection was based on OFV computed by NONMEM, goodness-of-fit graphical analysis and physiological plausibility. A ΔOFV of 3.84, 6.63 and 10.8 are considered significant at p=0.05, p=0.01 and p=0.001, respectively, with one degree of freedom.

One-, two-, three-, and four-compartment distribution models with elimination from the central compartment were considered. First-order, parallel first-order, zero-order, mixed first- and zero-order, and sequential zero- and first-order absorption models with and without an absorption lag-time were evaluated. A transit compartment model for the description of the absorption phase was also tried with a step-wise addition of compartments to find the optimal number of transit compartments in the population (38-39). Inter-individual random variability in all parameters was modelled exponentially. Oral bioavailability of piperaquine is affected by concomitant food intake and was therefore fixed to 100% but allowed for inter-individual variability since food intake was not controlled in the study. Inter-occasion variability was not considered since plasma concentration data were collected only after the last dose. Additive, proportional and intercept-slope error models were evaluated.

Collected continuous covariates (body weight, age, axillary temperature at admission, parasitemia at admission, and haemoglobin at admission) were modelled as linear and piecewise linear functions centred on the median value of the population. The influence of body weight was also investigated by an allometric function with clearance values scaled to mass to a power of 0.75 and with volume values scaled to mass to the first power (e.g. individual clearance value = typical clearance value × (individual body weight/median body weight in the population)$^{0.75}$) . Gender was evaluated as a dichotomous covariate. A stepwise covariate addition ($p<0.05$) and backward elimination ($p<0.001$) approach was automated using PsN.

Goodness-of-fit model diagnostics were performed and the extent of individual parameter estimate shrinkage (eta shrinkage) and epsilon shrinkage was calculated to assess the reliability of the goodness-of-fit diagnostics (40). Numerical and prediction-corrected visual predictive checks were used to evaluate the simulation properties of the model (41). The final model with estimated variability was used to simulate 2,000 concentrations at each of the individual sampling times and the 95% confidence intervals around the 5%, 50% and 95% percentiles of the simulations were overlaid with the 5%, 50% and 95% percentiles of observed data. A nonparametric bootstrap analysis of 1,000 iterations was performed for the final model. Monte Carlo simulations (1,000 simulated patients per kilogram body weight) by use of the final model with the observed variability were performed for different body weight-based dosing regimens to

obtain representative population estimates (median ± inter-quartile range) of day 7 piperaquine concentrations after a prospective dose regimen.

Pharmacodynamic analysis

Group comparisons were performed using a non-parametric Mann-Whitney test in STATA version 10 (Stata Corp., Texas, USA). A p-value of less that 0.05 was assumed to be statistically significant unless otherwise indicated. The risk of a new or recrudescent infection during the 42 day follow-up period was calculated as a proportion using STATA. A Cox regression analysis in STATA was used to evaluate the influence of covariates (age, body weight, parasitemia, individually predicted day 7 plasma piperaquine concentration and total piperaquine exposure) on new malaria infections during follow-up. Different distributions of the hazard function were evaluated (i.e. Weibull, exponential, log-normal, log-logistic, Gompertz, and generalized gamma distributions) and compared with a proportional-hazards function by visual inspection of the Cox-Snell residuals.