ORIGINAL ARTICLE

Dose selection of chloroquine phosphate for treatment of COVID-19 based on a physiologically based pharmacokinetic model

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Abstract Chloroquine (CQ) phosphate has been suggested to be clinically effective in the treatment of coronavirus disease 2019 (COVID-19). To develop a physiologically-based pharmacokinetic (PBPK) model for predicting tissue distribution of CQ and apply it to optimize dosage regimens, a PBPK model, with parameterization of drug distribution extrapolated from animal data, was developed to predict human tissue distribution of CQ. The physiological characteristics of time-dependent accumulation was mimicked through an active transport mechanism. Several dosing regimens were proposed based on PBPK simulation combined with known clinical exposure—response relationships. The model was also validated by clinical data from Chinese patients with COVID-19. The novel PBPK model allows in-depth description of the pharmacokinetics of CQ in several key organs (lung, heart, liver, and kidney).
1. Introduction

Coronavirus disease 2019 (COVID-19) which was declared a global pandemic by World Health Organization (WHO), has been spreading rapidly across the world, affecting more than 200 countries and claiming more than 700,000 confirmed cases. Approximately 20% of the patients with COVID-19 experienced fatal complications, including tissue failure, septic shock, pulmonary edema, severe pneumonia, acute respiratory distress syndrome (ARDS), and mortality rate among this population was estimated to be 50%.

Chloroquine (CQ) was first shown to effectively suppress Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in in vitro assay, and has been subsequently suggested to be efficacious in slowing the deterioration of pneumonia, improving lung imaging results, decreasing viral load, and thus shorten disease duration. CQ phosphate has been used for the treatment of malaria and autoimmune diseases for more than 70 years. According to the prescribing information, the dosage on the first day is not to exceed 1500 mg CQ phosphate, followed by daily maintenance dose of not exceeding 1000 mg CQ phosphate. Although CQ has acceptable safety profile, there are some potential safety concerns with prolonged usage, including QT prolongation, ventricular tachycardia, and retinopathy.

Studies revealed that CQ also had potential broad-spectrum antiviral activities by increasing endosomal pH being required for virus/cell fusion to accumulate in the cell, and interfering with the glycosylation of cellular receptors of SARS-CoV. It was reported that the angiotensin-converting enzyme 2 (ACE2) receptor, which SARS-CoV-2 employs for the entry into the cell, is highly expressed in lung, gastrointestinal tract, kidney, and heart, etc., which allows SARS-CoV-2 to easily enter these organs. CQ was reported to be highly and slowly accumulated in these organs. Therefore, the distribution of CQ in these organs could be highly relevant to its potential effectiveness against SARS-CoV-2 and adverse events. Following the currently recommended dosing regimen for treatment of malaria or rheumatoid arthritis, it is likely that drug concentration at the site of action is exceedingly higher than the efficacious concentration (EC50) needed to suppress the SARS-CoV-2 in vitro. Meanwhile, higher tissue accumulation of CQ may lead to adverse events. Therefore, dose of CQ should be optimized by considering exposure–efficacy and exposure–safety relationships of CQ. Among the infected patients, there are approximately 30% elderly, 30% with other complications such as hypertension and diabetes, as well as pregnant women and children (approximately 2%) population often presents as a challenge for health care providers in the clinical setting. Therefore, there is an urgent need to develop an individualized dosing strategy for each vulnerable population for the safe and effective use of CQ phosphate against SARS-CoV-2.

Physiologically-based pharmacokinetic (PBPK) model is an important mathematical tool that incorporates pharmacokinetic properties of drug and physiology, and allows the simulation of pharmacokinetic profiles of drug in plasma as well as other organs and tissues, including the site of action. It can also be used to predict drug PK in different patient populations under different treatment regimens. In Feb 2020, we employed an initial CQ PBPK model to simulate systemic and lung concentrations to support dosing recommendations of a clinical study in Wuhan (ChiCTR2000029898, ChiCTR2000029899). The initial CQ PBPK model was based on an earlier model developed by Certara UK (Simcyp Division) in collaboration with the Bill & Melinda Gates Foundation (Seattle, WA, USA) and Medicines for Malaria Venture, and is freely available within a Global Health PBPK model repository (https://members.simcyp.com/account/globalHealthRepository/). In addition, there is another report for Zika virus infection during pregnancy, where the PBPK model of chloroquine was established and validated by clinical blood and plasma time–concentration profiles. Neither of the above two models reported the model construction or predictions in tissues. As the distribution of CQ in tissues could be highly relevant to its potential effectiveness against SARS-CoV-2 and adverse events, our study aims to update the initial a PBPK model of CQ to i) understand the drug exposure in various tissues under different treatment regimens of CQ phosphate, ii) use the model to predict the drug concentration at the site of action as well as in the tissues of interest where toxicity is of concern, and iii) subsequently support dose selection in different patient populations infected with SARS-CoV-2. We also reported the pharmacokinetic data of Chinese patients with COVID-19 for the first time to validate the PBPK model.

2. Methods

2.1. Data collection

The physicochemical characteristics and pharmacokinetic parameters of chloroquine were collected in Pubmed and Embase database through literature research. Among these parameters, pKa, Log P, and Bi/P ratio were generated from in vitro experimental data. The fractional contribution of renal elimination and CYP2C8 and CYP3A4 were derived from public results. The permeability coefficient of chloroquine in human lung adenocarcinoma-3 (Calu-3, parameter relevant for predicting lung drug concentrations) cells was predicted by the QSAR model built in Simcyp software (Version 18, Certara, UK).
All clinical pharmacokinetic data of chloroquine were collected from Pubmed and Embase databases. The key words used for the search were “Clinical Pharmacokinetic and Chloroquine”. The publications from January 1, 1940 to February 29, 2020 were reviewed. At similar dose levels and comparable patient population, the blood drug concentration of chloroquine is significantly higher or lower than the observed values of similar studies by five-fold or more would be excluded. When pharmacokinetic parameters were not available, data were obtained from the concentration–time profiles in the publications by Plot Digi-tizer (GetData, Version 2.26), and were applied in Phoenix (Version 8.6, Certara, UK) to calculate the corresponding main PK parameters (area under curve, AUC and maximum drug concentration, C<sub>max</sub>).

### 2.2. Development of PBPK model

Simcyp (Version 18, Certara, UK) software was used to develop the PBPK model of CQ. The initial chloroquine PBPK model was developed by Certara UK (Simcyp Division) in collaboration with the Bill & Melinda Gates Foundation (Seattle, WA, USA) and Medicines for Malaria Venture.

A first-order absorption model with the input of f<sub>a</sub> and k<sub>a</sub> (fraction absorbed and first-order absorption constant, respectively) was used to describe the drug absorption process; a full-PBPK model was used to describe the drug distribution characteristics; the enzyme kinetic data and renal clearance data were used to describe the elimination characteristics; a model of permeability-rate limited mechanism was used to predict the pharmacokinetics of chloroquine in lung, heart, liver, and kidney tissues, and the perfusion-limited model was assumed in other organs/tissues.

Intracellular CQ accumulation was characterized by the inclusion of efflux and uptake mechanisms through undefined transporters in lung permeability-rate limited tissue compartments, with passive diffusion clearance (CL<sub>pd</sub>) optimized based on the ratio of time varying tissue-to-plasma concentration ratio (K<sub>e</sub>) and the ratio of the elimination half-life of chloroquine in tissues to that of plasma (R<sub>t</sub>) reported in rat. Because heart, liver, and kidney have high expression of ACE2, and CQ is known to cause cardiac toxicity, we established separate additional models (compound files) of CQ each representing heart, liver, or kidney with an “user defined additional organ” to explore drug accumulation dynamics. For each of these additional models, K<sub>e</sub>, R<sub>t</sub>, observed in rats and reported V<sub>in</sub> in human of 137.8 L/kg were used to optimize uptake and efflux mechanisms by undefined transporters.

### 2.3. Validation of PBPK model

The Simcyp Simulator trial design was set to match population demographics (including ethnicity, age, and sex), as well as the dosing and blood collection time points of each literature report. Each simulation includes 10 trials with 10 subjects in each trial. Simulated AUC and C<sub>max</sub> were compared with clinical observations to assess the predictive performance of the PBPK model. Evaluation criteria are: 1) the observed value is within the 90% confidence interval of the predicted value; 2) the ratio of simulated AUC and C<sub>max</sub> values are within 2-fold namely, 0.5 ≤ R ≤ 2.0 of the observed values. The use of a tighter boundary (within 25%) was also examined.

Two methods were applied to support the use of PBPK model for predicting tissue drug concentrations: 1) the ratio of time dependent K<sub>e</sub> of CQ in rats<sup>23</sup> and predicted K<sub>e</sub> in human; and 2) the ratio of the elimination half-life of CQ in R<sub>t</sub> observed in rats<sup>23</sup> and predicted R<sub>t</sub> in human by PBPK model. Evaluation criteria are: 1) overlapping of the prediction profiles in human and observation profiles in rats and comparison of the variability of K<sub>e</sub> in rats and the predicted variability of human K<sub>e</sub>; 2) The variation range of the calculated R<sub>t</sub> value should be within 2-fold, namely, 0.5 ≤ R ≤ 2.0.

### 2.4. Dosage regimen design and simulation

According to the preliminary clinical data from novel COVID-19 patients<sup>2</sup>, the dosing schedule of 500 mg BID for 7 days was recommended in the Guidelines (7th edition) for the Prevention, Diagnosis, and Treatment of Pneumonia Caused by COVID-19 issued by the National Health Commission of the People’s Republic of China. Assuming this dose regimen is effective, we chose to predict the trough concentration in lung tissue on the fifth day (at post-dose 120 h) as an indicator of the minimum efficacious concentration, and the predicted lung tissue AUC<sub>Lung</sub> as the minimum efficacious exposure level. In addition, safety limits and warning limits were set based on the dose—safety relationship of CQ in the rheumatoid arthritis patients.<sup>24</sup>

According to the approved dosage in product label, the standard antimalarial treatments (Regimen A), the highest dose that demonstrates symptom improvement in the treatment of rheumatoid arthritis (Regimen B) and the apparent effective clinical treatment (Regimen C) were selected as reference regimens. Using PBPK model, we simulated PK profiles of CQ under these reference regimens and overlaid predictions of the above efficacious and safety concentrations. These simulations guided us to propose three dosage regimens individualized for the following patients with COVID-19: acute patients (Regimen D), moderate patients (Regimen E) and special populations (Regimen F). The simulated populations include Chinese healthy volunteers, children (0–17 years old), pregnant women (in second trimester), elderly (65–98 years old) and patients with hepatic and renal impairment. Unless otherwise stated, default models within SimCYP population library were used. Ratio of male to female subjects was set to 1:1 for all simulations.

### 2.5. Clinical pharmacokinetic study of COVID-19 patients

An open-label, single-center study (Ethical review approval number: PJ-NBEE-YK-2020-063-01) was conducted to assess the safety, efficacy, and pharmacokinetics of CQ in patients with COVID-19. A total of eight patients weighing more than 60 kg were orally given 500 mg CQ phosphate BID for 7 days (same with Regimen C). Plasma samples on Day 1, Day 3, Day 5, Day 7 and Day 14 were collected prior to dose administration. The plasma concentrations of CQ were determined using a validated high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method (see details in Supporting Information). The study was approved by the Ethics Committee of Ningbo Hwamei Hospital, University of Chinese Academy of Sciences (Ningbo, China), and was performed in accordance with the Declaration of Helsinki. All subjects signed the Informed Consent Form (ICF) before the study.
3. Results

A PBPK model of CQ incorporating in-depth tissue distribution in the lung was developed and validated using CQ PK data from human blood and plasma, and tissue distribution informed from reported rat plasma and tissues. With this model, we can propose an appropriate dose optimization strategy to treat COVID-19 in acute patients, moderate patients, and special vulnerable populations who may need dose adjustments. The overall model-informed dosing strategy is shown in Fig. 1.

3.1. Development of PBPK model

The final model parameters and sources are shown in Table 1. A total of 28 articles related to human pharmacokinetics of chloroquine were collected based on the search criteria. There were four articles that were excluded according to the exclusion criteria, and a total of 39 CQ concentration-time profiles from different populations following administration of CQ phosphate were included (the retrieval method is shown in Supporting Information Fig. S1. Of these studies, one was used to build the model25, and the remaining 38 profiles were used to validate the model. The population characteristics of all drug concentration-time curves and the design of dosing regimens are shown in Supporting Information Table S1.

3.2. Validation of PBPK model

The 38 concentration-time profiles from different clinical studies were compared with the predicted blood or plasma concentrations to verify the predictability of the PBPK model. The results show that 94% (31/33) of the observed AUC values were adequately described by PBPK simulations within 0.5–2.0-fold, and 45% (15/33) of observed AUC values were described by PBPK simulations within 0.8–1.25-fold. Regarding C_max, 97% (32/33) observed C_max values were described by PBPK simulations within 0.5–2.0-fold, and 18% (6/33) observed C_max values were described by PBPK simulations within 0.8–1.25-fold. Validation results are shown in Fig. 2, which includes data used for model building25.

3.3. Dosage regimen design and simulation

There are total of six regimens of CQ phosphate investigated in this study (Table 3). PBPK model was used to simulate regimens A–E in healthy Chinese subjects. Simulation of Regimen F was tested in Chinese healthy volunteers, Geriatric Northern European Caucasians (NEC), cirrhosis (mild, moderate and severe), renal glomerular filtration rate (GFR, 30–60 mL/min/1.73 cm² and less than 30 mL/min/1.73 cm²), pregnancy (in second trimester) and pediatric populations default in Simcyp (Version 18.0).

The basis for dose selection is described as follows. Regimen C has been clinically used as an apparent efficacious dose5. The PBPK model predicted trough concentration in the lung tissue under Regimen C on Day 5 is 60.6 µg/mL, and the predicted total exposure for five consecutive days (AUC₀⁻¹₂₀ h) is 3020 h µg/mL. These two values are defined as the effective concentration and the effective exposure, respectively. Based on Frisk-Holmberg et al.24, the peak serum concentration with almost no adverse reactions was 400 ng/mL, so it was used as a safety limit (similar to a no-adverse effect level). The warning limit was set at a maximum serum concentration of 800 ng/mL, at which adverse effects were observed in approximately 80% subjects. Assuming plasma concentrations are equivalent to serum concentrations, we used PBPK model to simulate plasma and lung tissue concentrations of CQ under six different regimens (Table 3). Simulated drug concentration-time curves in plasma, blood, and lung tissue are shown in Fig. 4.

The simulation results show that the predicted population mean C_max of the conventional clinical treatment Regimens A and B were below 400 ng/mL, which was consistent with clinical evidence for the approved indications. Regimen C mimics clinical...
study in which apparent efficacy in COVID-19 patients was observed\textsuperscript{5}. Under this regimen, the model simulated population mean $C_{\text{max}}$ of plasma exceeded 400 ng/mL, but was substantially below 800 ng/mL.

Regimen D was designed for treating patients with acute COVID-19. Under this regimen, the model-simulated population mean trough concentration in lung tissue could reach 60.6 mg/mL in 3–5 days. Regimen E was designed for treating moderate COVID-19 infected patients, and model simulated mean trough concentration in lung tissue can reach 60.6 mg/mL in 5–7 days. The total exposures ($AUC_{0-120}\ h\ mg/mL$) for five consecutive days of both Regimens D and E (3650 and 3220 h $\mu$g/mL, respectively) are greater than 3020 h $\mu$g/mL. The total exposures ($AUC_{0-240}\ h$) for 10 consecutive days of Regimens F ($6470\ h\ \mu$g/mL) is about twice as much as 3020 $\mu$g/mL. When the simulated concentration–time profile is extended to 28 days, the result shows that the elimination of the drug in the body is slow and drug accumulation in the tissues is high. For example, in Regimen C,

| Parameter | Input value | Source |
|-----------|-------------|--------|
| Molecular weight (g/mol) | 319.87 | Calculated from measured LogD$_{7.4}$ = 0.93\textsuperscript{18} |
| $\log P$ | 4.37 | Measured\textsuperscript{18} |
| Compound type | Diprotic base | Assumed |
| $pK_a$ | 9.94, 8.40 | Assumed |
| Blood-to-plasma partition ratio | 3.50 | Measured\textsuperscript{18} |
| Hematocrit | 45.0 | Assumed |
| Fraction unbound in plasma | 0.40 | Measured\textsuperscript{33,34} |
| Absorption | | |
| Absorption model | First-order | Assumed |
| Fraction available from dosage form ($f_a$) | 1 | Optimal via sensitivity analysis for $T_{\text{max}}$ about 4 h |
| Absorption rate constant ($k_{a,h}/C_0$) | 0.50 | |
| Unbound fraction of drug in enterocytes ($f_u\text{Gut}$) | 1 | Assumed |
| Polar surface area ($\AA^2$) | 25.7 | |
| Distribution | | |
| Distribution model | Full PBPK model | Method 2 plus $K_p$ scalar optimized to recover the observed to equal mean observed human $V_{ss}$\textsuperscript{35,36} |
| $V_{ss}$ (L/kg) | 137.8 | |
| $K_p$ scalar | 2.755 | |
| Elimination | | |
| Clearance type | Enzyme kinetics | |
| $C_{\text{int}}$ of recombinant CYP2C8 ($\mu$L/min/pmol of isofrom) | 0.269 | Calculated using retrograde model with $f_e$ 0.5, $f_m$ CYP2C8 0.25, fmCYP3A4 0.15 and fm undefined 0.1\textsuperscript{19,20,37} |
| $C_{\text{int}}$ of recombinant CYP3A4 ($\mu$L/min/pmol of isofrom) | 0.0283 | |
| Additional clearance of HLM ($\mu$L/min/mg protein) | 2.58 | |
| Typical renal clearance $C_{\text{GL}}$ (L/h) | 20.76 | |
| Concentration of inhibitor that supports half maximal inhibition ($K_p, \mu$mol/L) CYP2D6 | 3.15 | |
| Fraction of unbound drug in the $in\ vito$ microsomal incubation ($f_{\text{mic}}$) | 0.797 | Calculated for 0.1 mg/mL from measured value of 0.496 at 0.4 mg/mL |
| Fraction unbound in pulmonary mass | 0.001 | |
| Number of hydrogen bond donors (HBD) | 1 | Drug bank |
| Fraction unbound in pulmonary mass | 0.001 | Optimized based on $K_p$ and $R_i$ in rat |
| An undefined, transporter-mediated clearance ($\mu$L/min/cm$^2$ for lung and $\mu$L/min $\times 10^6$ for granuloma) | 0.015 | |
| Whole organ passive diffusion clearance between intra- and extra-cellular water (L/h) | 0.1 | Optimized based on $K_p$ and $R_i$ in rat and $V_{ss} = 137.8$ (L/kg); user defined additional organ is permeability limited |
| Passive diffusion clearance for heart $C_{\text{liver}}$ (L/min) | 0.1 | |
| Uptake clearance for heart $C_{\text{liver}}$ (L/min) | 11,000 | |
| Efflux clearance for heart $C_{\text{liver}}$ (L/min) | 9 | |
| Passive diffusion clearance for heart $C_{\text{liver}}$ (L/min) | 0.1 | |
| Uptake clearance for liver $C_{\text{liver}}$ (L/min) | 59,000 | |
| Efflux clearance for liver $C_{\text{liver}}$ (L/min) | 9 | |
| Passive diffusion clearance for heart $C_{\text{kidney}}$ (L/min) | 0.1 | |
| Uptake clearance for kidney $C_{\text{kidney}}$ (L/min) | 34,000 | |
| Efflux clearance for kidney $C_{\text{kidney}}$ (L/min) | 10 | |

Abbreviations: $P$, octanol–water partition coefficient; PBPK, physiologically-based pharmacokinetic; $V_{ss}$, volume of distribution at steady-state; $K_p$, partition coefficient; HLM, human liver microsome.

\textsuperscript{a}Rodgers and Rowland prediction method was used.
plasma concentration on Day 28 reached 60.4 ng/mL, whereas predicted lung tissue concentration was 124 μg/mL. Simulations under these regimens also indicate high CQ concentrations in the heart, liver, and kidneys (Fig. 5, take Regimen F for example).

Regimen F was a reduced-dose regimen intended for other vulnerable populations with COVID-19. In these populations, exposure may be increased due to reduced drug elimination. Model simulated results show that CQ exposure in the elderly (65–98 years), and patients with hepatic and renal impairment were higher than in normal adults, but the exposure in pregnant women was lower as shown in Fig. 6. The results showing that children of different age groups could achieve similar plasma and blood exposure to adults by adjusting the dose regimen are shown in Fig. 7.

### 3.4. Clinical pharmacokinetic study of COVID-19 patients

A total of 25 plasma samples were collected and analyzed successfully from eight patients with moderate COVID-19. After multiple oral doses (500 mg BID, CQ phosphate) for 7 days, the mean concentrations were 169.4 ± 106.4, 322.1 ± 112.0, 245.4 ± 89.2, and 159.5 ± 93.1 ng/mL for Day 3, Day 5, Day 7, and Day 14, respectively. In accordance with this protocol, we compared the PBPK-model-predicted CQ concentrations in healthy Chinese volunteers to these observed data in patients with COVID-19, as shown in Fig. 8, and the results show that the predicted mean concentration was within 2-fold of the observed mean concentration, indicating that the pathological state might have little influence on the pharmacokinetic characteristics of drug in plasma.
4. Discussion

4.1. Dose optimization strategy for outbreak of COVID-19

At present, in many clinical studies (ChiCTR2000029939, ChiCTR2000029935, ChiCTR2000029899, etc.) conducted in China, there are no standard recommended dosing regimens of chloroquine phosphate (see Supporting Information Table S2). After preliminary clinical research and exploration, National Health Commission of the People’s Republic of China proposed that the recommended dose of chloroquine phosphate for the treatment of COVID-19 was 500 mg BID for adults (18–65 years), and continuous administration should not exceed 7 days. In clinical setting, the dose is often being adjusted case-by-case based on the experience of the clinicians, and the selection of optimal dosing regimen would require a well-designed clinical trial with prolonged time period for safety monitoring with large sample size. Under the current urgent need of the treatment to help patients with COVID-19, it is not feasible to conduct such conventional clinical trials to optimize dose selection for CQ. Therefore, we borrowed the principles of the US FDA’s Animal Rule and used PBPK modeling as a tool to combine the available cell-level and animal-level data and describe quantitatively the dose—exposure—response relationships of CQ. Modeling and simulation approach were employed to understand and translate the kinetics of CQ from the cell level to the whole-body level, and were used to support the design of dosing regimens for different patient populations.

4.2. Advantages of the updated PBPK model

PBPK model integrates a series of mathematical equations that describe human physiological and biochemical pathways, drug physicochemical properties and drug mechanism pharmacokinetic data parameters to systematically study the body’s effects on drugs. The model-based selection of dosing regimens of CQ presented in our study was based on the following knowledge: in vitro antiviral potency, historical knowledge of exposure—response relationship for safety, apparent efficacy reported recently (e.g., under dosing Regimen C), and a PBPK model capable of predicting drug concentrations in plasma, blood, and tissues. The novelty of this study includes (i) mimicking the physiological characteristics of time-dependent accumulation through an active transport mechanism in the model, which reasonably captured high accumulation of chloroquine in the cells for better prediction of the local PK characteristics of CQ.
in permeability-limited tissues, and (ii) supporting PBPK prediction of human tissue drug concentrations using animal data. The collection of the lung tissue sample presents a challenge in clinical operation. Considering that the accumulation of CQ in tissues was mainly due to passive diffusion and increased intracellular pH, we assumed that there is minimal inter-species difference. It is thus reasonable to use the rat $K_p$ and $R_t$ values to support the prediction of tissue concentrations using PBPK models. In the absence of clinical experimental data, this is a powerful alternative to predict the high $K_p$ ratios of human according to observations in rats.

As ACE2 receptor is highly expressed in liver, kidney, and heart, which allows SARS-CoV-2 virus to easily enter these

Figure 4  Simulation results of six dosage regimens. (a) Plasma concentration for 10 days; (b) plasma concentration for 28 days; (c) blood concentration for 10 days; (d) blood concentration for 28 days; (e) lung concentration for 10 days; (f): lung concentration for 28 days. Additional models (SimCYP compound files) for heart, liver, or kidneys, respectively, were used to simulate tissue concentrations (See Methods).

Figure 5  Simulation results of six dosage regimens. (a) Heart concentration for 28 days; (b) liver concentration for 28 days; (c) kidney concentration for 28 days.
accumulation of CQ in these organs could be highly relevant to its potential effectiveness. Meanwhile, significant drug accumulation in these organs may raise safety concerns. Considering it could passively enter tissue cells and can be trapped in some organelles in the ionized form \(^{22}\), permeability-limited distribution was assumed in these tissues to characterize the time-dependent cellular drug accumulation. The simulated results showed that CQ could retain in tissues for a long time after dosing stopped.

4.3. Considerations of dosing regimen for special population

In order to apply right dosing regimen to special populations, we performed model simulations to assess the relationship of drug exposure and safety. The liver metabolic enzyme activity and glomerular filtration rate of CQ in these special populations are altered \(^{29-32}\). However, as CQ was almost equally eliminated by CYP450-mediated metabolism and renal secretion \(^{13}\), dramatic change in either pathway theoretically might not lead to significant increase in CQ exposure, as shown by our simulation results. The results suggested that there is no drastic increase in exposure for the elderly, patients with renal or hepatic impaired functions, or pregnant women. In these subjects, dose adjustment can be used according to proposed regimens based on exposure-matching. Considering known safety and emerging information on efficacy, we proposed the use of Regimen F as starting regimen for treating COVID-19 in these special populations. However, in other sensitive populations, such as children with different ages or the patients with both of impaired renal and hepatic functions, CQ exposure may increase dramatically, and CQ should be avoided or

**Figure 6** Simulation results of special populations with Regimen F. (a) Plasma concentration for 28 days; (b) blood concentration for 28 days; (c) lung concentration for 28 days.
used very cautiously if it has to be used. For example, a regimen with a lower loading dose and less frequent dosing may be considered. The developed drug model and population models can be used to predict the exposure and design different dosage regimens. Linking the exposure with clinical effect, the dose of CQ in special population can be properly guided.

4.4. Limitations of the research

Multiple assumptions were made in this study and several limitations warrant additional research to further enhance the predictability of the model. First, we assumed same underlying physiology between healthy subjects, patients with acute or moderate COVID-19 and virtual populations that better represent COVID-19 disease related pathophysiology may be needed. For example, although simulated mean CQ concentrations were within 2-fold of the observed concentrations, observed results are highly variable, and simulated mean concentration appears to underestimate and overestimate observed values on Day 4 and Day 6, respectively (Fig. 8). Disease progression during intervention of CQ may affect the PK of the drug. Sensitivity analysis was performed on the newly established model, such as the change of free fraction of CQ in lung tissue, pulmonary blood flow, pulmonary pH, and intrinsic clearance rate (CLint) of lung tissue. The results suggest that these physiological and pathological changes had little impact on the predictions (data not shown). Second, simulations were conducted in various virtual populations that have been developed based on Caucasian data. The predictability of CQ pharmacokinetics in vulnerable populations may require further validation. Third, we focused on liver, lung, heart, and kidney when parameterizing permeability-limited drug distribution. Future research is needed to characterize drug distribution to other organs such as the eyes. CQ is known to cause retinopathy, understanding tissue distribution in this organ is important. In addition, several assumptions were made when parameterizing permeability-limited CQ distribution, including the use of apparent active transport mechanism to capture time-dependent drug accumulation, the use of animal data to support prediction of tissue concentrations in humans, and the use of additional models and undefined extra organ to describe permeability-limited tissue distribution in the heart, liver, and kidney. Last but not the

![Figure 7](image-url) Simulation results of children with Regimen F. (a) Plasma concentration for 28 days; (b) blood concentration for 28 days.

![Figure 8](image-url) Simulated plasma concentration time profiles of CQ in Chinese healthy subjects overlaid with observed plasma concentration time profiles of CQ in Chinese COVID-19 patients (500 mg BID for 7 days, CQ phosphate). Solid lines represent mean simulated concentration time profile with dotted lines representing 5th and 95th percentile range. Red circles represent observed clinical data from COVID-19 study. Where presented, error bars indicate standard deviation, n = 8.
least, it has to be noted that discussion on exposure-efficacy relationship discussed in this manuscript is exploratory as several assumptions were used. In general, the concentration of free plasma should be compared with EC50. Considering the high accumulation of this drug in cells, it should be valuable to compare intracellular EC50 with lung tissue concentration. In in vitro experiment16, we observed time-dependent decrease in EC50 values, and intracellular drug accumulation over time may be one of the causes (e.g., higher concentration after longer incubation). This may be modified when we can truly measure intracellular CQ concentrations in vitro and in vivo and then compare intracellular EC50 with lung tissue concentration. We explored the use of in vivo efficacious concentration with assumption that anti-viral outcome from a recommended dosage regimen for COVID-19, which should be further confirmed by a strict randomized and well-controlled trial. If the regimen of 5th day of 500 mg BID confirmed to be modified, then the final plan also needs to be optimized, but the strategy is similar.

5. Conclusions

We proposed a model-informed dose selection strategy under emergency situation. First, we established and validated a novel PBPK model to predict concentrations in lung, heart, liver and kidney assuming permeability-limited distribution by mimicking the physiological characteristics of time-dependent accumulation through an active transport mechanism in the model. Second, we selected the simulated lung trough concentration on Day 5 and AUC0–120h in patients with a dose of 500 mg BID (CQ phosphate) as effective target and selected 800 ng/mL of plasma trough concentration as safety limit according to reported clinical exposure–response relationship. Third, we optimized different dosing regimens for different type of patients using PBPK model. Fourth, pharmacokinetic data from Chinese patients with moderate COVID-19 was firstly reported and employed to validate the model. Recognizing the limitations and assumptions, we are presenting a mechanism-based, rationalized modeling approach to healthcare providers to facilitate their decisions on dose selection. We hope our PBPK model could be applied for optimizing the use of CQ for treatment of COVID-19, and our dosing optimizing strategy could help accelerate safe and effective use of other anti-COVID-19 drugs.

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Author contributions

Dongyang Liu conceived and designed research; Haiyan Li direct the whole project; Shun Zhang designed the pharmacokinetic study; Cheng Cui, Miao Zhang and Xuetong Yao developed and optimized the tissue models; Siqi Tu, Zhe Hou, and Valerie Sia Jie En performed data collection and model in vitro; Jing Lin and Ting Cai assayed the pharmacokinetic data. Xiaoliang Xiang, Ning Shen, Chunli Song and Jie Qiao provided valuable suggestions; Cheng Cui wrote the paper.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting data related to this article can be found at https://doi.org/10.1016/j.apsb.2020.04.007.

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