Pharmaceutical Sciences Asia

Pharm Sci Asia 2021; 48(5), 425-431

DOI:10.29090/psa.2021.05.21.002

Research Article

Optimized dosing regimen of hydroxychloroquine for treatment of coronavirus disease 2019 using Monte Carlo simulation

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ABSTRACT

Optimized dosage regimens of hydroxychloroquine (HCQ) in coronavirus disease 2019 (COVID-19) are currently unknown. We aimed to determine regimens that rapidly achieved the pharmacokinetic-pharmacodynamic (PKPD) target for virological clearance in COVID-19 patients. Plasma HCQ concentration was simulated using a non-steady state, 2-compartment linear model. The plasma trough concentration (C_{trough}) ≥ 0.7 mg/L was used as the PKPD target. The loading dose of 800 mg three times daily and 1,200 mg twice daily achieved the target on the first day with the probability of target attainment (PTA) 97.53% and 82.63%, respectively. Maintenance dose of 200 mg three times daily and 400 mg twice daily provided PTA > 80% from day 3 through day 10 after the initiation of HCQ therapy. All proposed regimens had the PTA < 1% to achieve toxic level of 4 mg/L. The optimal dose regimens for early viral clearance in COVID-19 patients were HCQ 800 mg three times daily on the first day followed by 200 mg three times daily for 9 days, and HCQ 1,200 mg twice daily on the first day followed by 400 mg twice daily for 9 days. Further clinical study is needed to ensure clinical efficacy and safety of these regimens.

Keywords:
Coronavirus; COVID-19; Hydroxychloroquine; Monte Carlo simulation; SARS-CoV-2

1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an outbreak of acute respiratory illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Fourteen percent of patients developed severe illness requiring oxygen therapy and approximately 5% have multi-organ dysfunction requiring intensive care unit (ICU) treatment[1]. Multiple host factors were associated with severity including age ≥ 50 years, male sex, cardio-vascular disease, diabetes, and malignancy[2]. However, there have not been any medications or other therapeutic options presently approved by the U.S. Food and Drug Administration (FDA)[3]. Hydroxychloroquine (HCQ) is currently being distributed to selected hospitals by the government in several countries, based on *in vitro* data and clinical studies showing potential benefits[4-6]. HCQ effectively inhibits viral replication by elevating the pH of endosomes and lysosomes[7]. Their anti-inflammatory properties that were demonstrated in the treatment of autoimmune diseases, may also reduce the inflammatory response to viral infection[8].

Currently, there is no conclusive evidence to support the optimal dosing and duration of HCQ for the treatment of COVID-19. Several HCQ regimens have been proposed in *in vitro* studies including 400 mg twice daily followed by 200 mg daily for 4 days[7]. In Thailand, the current national guideline recommended the regimen of 600 mg twice daily on the first day followed by 400 mg daily for 4-9 days[6]. However, these regimens have not been

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evaluated in the clinical study[6,7].

In a small, open-label, non-randomized clinical trial (RCT) conducted in mild COVID-19 patients using HCQ 200 mg three times daily showed the negative results for SARS-CoV-2 RNA by polymerase chain reaction (PCR) from the nasopharyngeal swab, indicating the virological clearance was achieved within 3-6 days of the treatment[9]. In contrast, the same dose of HCQ did not show virological clearance during 5-6 days of the treatment in an observational study because most patients had significant comorbidities associated with poor outcomes[10]. A pilot study with the dose of HCQ 400 mg daily for 5 days found no difference in the rate of virological clearance at day 7 compared with placebo[11]. Also, in another open-label, RCT with the regimen of loading dose HCQ 1,200 mg daily for three days followed by a maintenance dose of 800 mg daily for 3-4 weeks showed no significant increase in the virological clearance rate at day 28 when compared with routine standard of care without HCQ[12].

Because of the conflicting results reported from the different dosing regimens of HCQ, the study to determine optimal HCQ dosing regimen that achieves favourable efficacy and less toxicity is needed. Monte Carlo simulation is a mathematical technique that randomly generates drug concentration based on a pharmacokinetic (PK) model and probability distribution of PK parameters. It is used for calculation of the probability of target attainment (PTA), which is the possibility of the selected dose regimens to achieve the pharmacokinetic-pharmacodynamic (PKPD) target[13, 14]. Based on *in vitro* SARS-CoV-2 viral load inhibition by HCQ[7], a rapid increase in HCQ concentration should theoretically result in an early decline in viral load and possibly increase rate of virological clearance. Thus, this study aimed to determine the optimal HCQ regimens that rapidly attained the PKPD target for virological clearance in COVID-19 patients, together with the possibility to achieve the toxic level.

2. MATERIALS AND METHODS

2.1. Pharmacokinetic model

Based on short-term treatment of HCQ in COVID-19, we employed a non-steady state PK data derived from a two-compartment linear model with first-order absorption and lag time from healthy subjects and malaria patients for simulation[15]. The population PK parameters used in this study are shown in Table 1. In addition, oral bioavailability (F) of HCQ was fixed to 0.746 referred from the previous study[16].

2.2. Pharmacodynamic model

PKPD target associated with virological clearance was found only in the open-label, non-RCT conducted in mild COVID-19 patients. At day 6 of treatment, patients receiving HCQ with negative PCR for SARS-CoV-2 RNA from nasopharyngeal swab had a mean serum HCQ concentration of 0.612 mg/L[9]. However, the patients with significant comorbidities associated with poor outcomes receiving the same dose of HCQ did not show virological clearance with mean trough concentration of 0.678 mg/L[10]. Besides, the study of HCQ determination in systemic lupus erythematosus patients found that the serum concentration of HCQ is higher than plasma concentration with ratio of 0.54:0.44[17]. Therefore, we considered plasma trough concentration (C_{trough}) of 0.7 mg/L at any day of treatment as the minimum PKPD target for virological clearance. The simulated plasma maximum concentration (C_{max}) of the lowest reported toxicity after a single dose ingestion of 4 g HCQ, was used as a cut-off level for toxicity[18].

2.3. Monte Carlo simulation

The mean values of PK parameter and inter-individual variability (IIV) from the final population PK model were used to simulate HCQ plasma concentration of 10,000 subjects during day 1-10 using Monte Carlo simulation (Crystal Ball 2017 v.2.2; Decisioneering Inc., Denver, CO). There were 8 regimens chosen from *in vitro* study, clinical studies, Thai national guideline, along with our proposed regimens, were simulated (Table 2). Regimens with high loading doses were expected to rapidly achieve the PKPD target. Log-normal distributions were set for between-patient variability. PTA was calculated as the percentage of all 10,000 estimates which achieve the target plasma $C_{trough} \geq 0.7$ mg/L. Regimen yielding PTA of > 90% is considered optimal[13]. PTA of the toxic level was also calculated to assure the safety of simulated regimens.

3. RESULTS

The simulated plasma HCQ concentrations of different regimens are shown in Figure 1. There were six regimens (regimen 3, 4, 5, 6, 7, and 8) that could reach the target plasma C_{trough} of 0.7 mg/L within 10 days of therapy. However, only two regimens (regimen 7 and 8) could reach the target since day 1.

The PTA analyses of various HCQ regimens are shown in Table 3. For published regimens, regimen 1 and 2 could not achieve PTA target of $C_{trough} \geq 0.7$ mg/L; but regimens 3, 4, and 5 achieved 90% PTA on day 10, 7, and 7, respectively.

For our proposed regimens, regimen 6 achieved 90% PTA of $C_{trough} \geq 0.7$ mg/L on day 6. Loading dose of 800 mg three times daily on the first day in regimen 7 provided PTA of 97.53%, while 1,200 mg twice daily on the first day in regimen 8 provided PTA of 82.63%.

Maintenance dose of regimen 7, and 8 provided PTA > 80% from day 3 through day 10 after the initiation of HCQ therapy.

Simulated C_{max} from a single toxic dose of 4 g was 4 mg/L. Once daily dosing in regimen 5 attained > 1% PTA of $C_{max} \geq 4$ mg/L since day 7, but all other regimens had the PTA < 1% over 10-day course of HCQ therapy. Increasing frequency to three times daily in regimen 7 increased the PTA of $C_{trough} \geq 0.7$ mg/L, reduced the PTA of $C_{max} \geq 4$ mg/L, and help reduce total maintenance dose per day.

Table 1. Estimate population pharmacokinetic parameters[15].

Parameters	Units	Estimate value	IIV	SD*
k_a	hr^{-1}	1.15	N/A (fixed at 0)	N/A (fixed at 0)
ALAG	hr	0.3890	0.036	0.074
Vc	L	437	0.232	210.487
Vp	L	1,390	0.715	1175.352
Q	L/hr	45.1	N/A (fixed at 0)	N/A (fixed at 0)
CL/F	L/hr	10.9	0.161	4.374

*Standard deviation = Estimate value $\times \sqrt{IIV}$

IIV = interindividual variability, k_a = absorption rate constant, ALAG = absorption lag time, Vc = central volume of distribution, Vp = peripheral volume of distribution, Q = intercompartmental clearance, CL/F = apparent clearance

Table 2. Hydroxychloroquine dosage regimens for simulations.

Dose regimens	References
400 mg OD for 5 days	Chen et al.[11]
400 mg BID day 1, then 200 mg BID for 4 days	Yao et al.[7]
200 mg TID for 10 days	Gautret et al.[9], Molina et al.[10]
600 mg BID day 1, then 400 mg BID for 9 days	Thai national guideline[6]
1,200 mg OD day 1-3, then 800 mg OD for 3-4 weeks	Tang et al.[12]
800 mg BID day 1, then 400 mg BID for 9 days	
800 mg TID day 1, then 200 mg TID for 9 days	Our study regimens
1,200 mg BID day 1, then 400 mg BID for 9 days	

OD = once daily, BID = twice daily, TID = three times daily

Table 3. Probability of target attainment for different hydroxychloroquine regimens with targets of $C_{trough} \geq 0.7$ mg/L for virological clearance in COVID-19 and $C_{max} \geq 4$ mg/L for toxic level.

%PTA	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
400 mg OD D1 - D5										
$C_{trough} \geq 0.7$ mg/L	0.00	0.14	1.77	6.40	13.42	5.81	2.43	1.02	0.40	0.23
$C_{max} \geq 4$ mg/L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
400 mg BID D1 then 200 mg BID D2 - D5										
$C_{trough} \geq 0.7$ mg/L	0.95	4.32	11.46	20.25	29.33	15.00	7.51	3.95	1.77	0.98
$C_{max} \geq 4$ mg/L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
200 mg TID D1 - D10										
$C_{trough} \geq 0.7$ mg/L	0.13	7.44	27.54	48.20	63.77	73.97	81.27	85.97	89.48	91.49
$C_{max} \geq 4$ mg/L	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01
600 mg BID D1 then 400 mg BID D2 - D10										
$C_{trough} \geq 0.7$ mg/L	10.12	36.68	60.99	75.72	84.65	89.58	92.76	94.88	96.26	97.24
$C_{max} \geq 4$ mg/L	0.00	0.00	0.01	0.01	0.02	0.05	0.11	0.20	0.43	0.66
1,200 mg OD D1-3 then 800 mg OD D4 - D10										
$C_{trough} \geq 0.7$ mg/L	6.07	45.43	73.04	80.38	86.19	89.73	92.40	94.17	95.29	96.23
$C_{max} \geq 4$ mg/L	0.02	0.29	1.06	0.46	0.65	0.86	1.38	1.74	2.30	2.89
800 mg BID D1 then 400 mg BID D2 - D10										
$C_{trough} \geq 0.7$ mg/L	32.46	52.20	70.26	81.35	87.53	91.25	93.83	95.43	96.59	97.40
$C_{max} \geq 4$ mg/L	0.00	0.00	0.00	0.01	0.08	0.15	0.21	0.29	0.45	0.81
800 mg TID D1 then 200 mg TID D2 - D10										
$C_{trough} \geq 0.7$ mg/L	97.53	74.06	79.84	84.86	88.61	91.25	93.15	94.67	95.68	96.57
$C_{max} \geq 4$ mg/L	0.10	0.01	0.01	0.01	0.02	0.03	0.06	0.07	0.08	0.09
1,200 mg BID D1 then 400 mg BID D2 - D10										
$C_{trough} \geq 0.7$ mg/L	82.63	74.27	82.73	88.12	91.77	94.15	95.69	96.76	97.48	98.00
$C_{max} \geq 4$ mg/L	0.41	0.04	0.07	0.11	0.17	0.24	0.41	0.64	0.90	0.98

PTA = probability of target attainment, OD = once daily, BID = twice daily, TID = three times daily, C_{trough} = trough concentration, C_{max} = maximum concentration

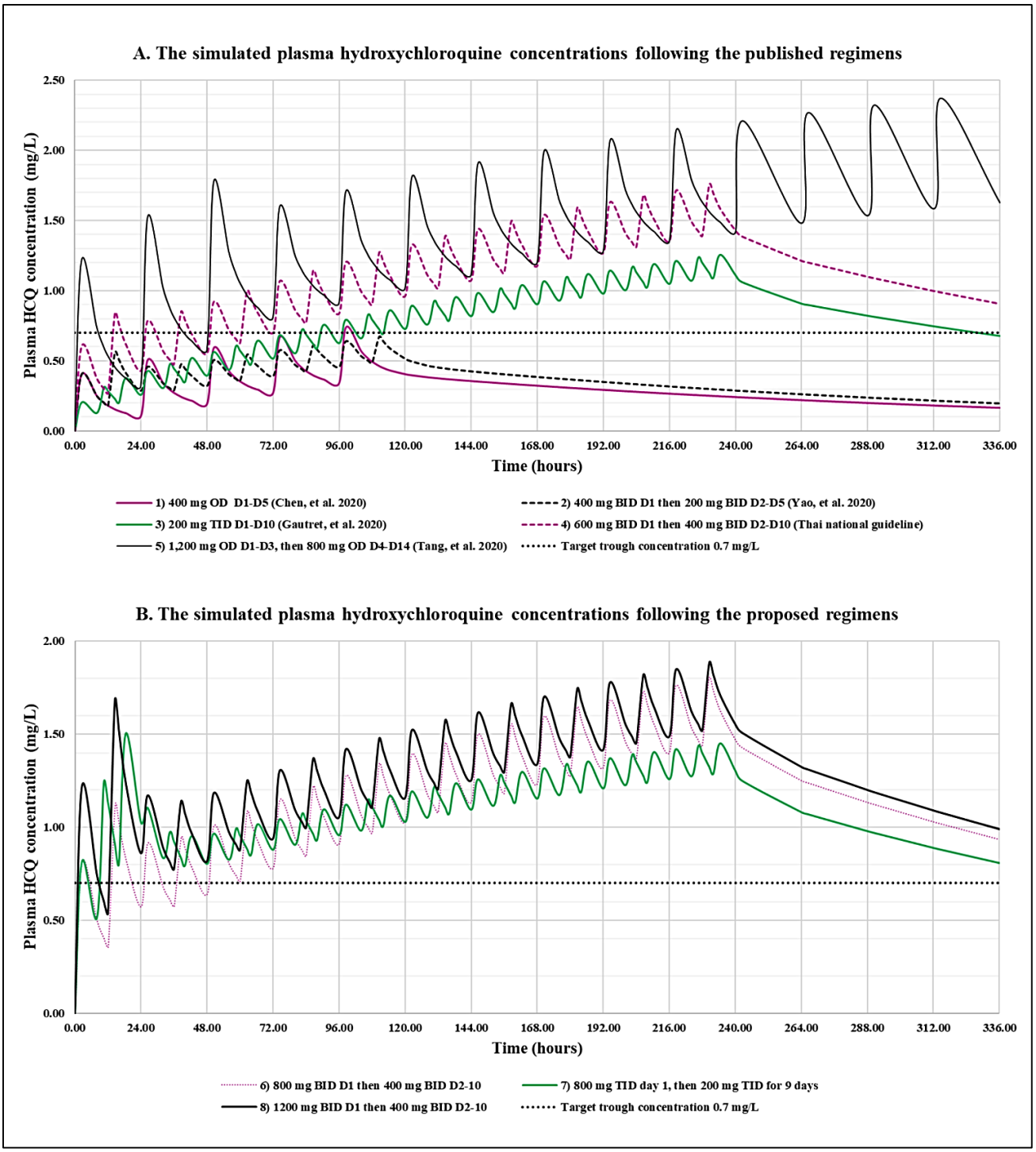

Figure 1. The simulated plasma hydroxychloroquine concentrations of different regimens. **A.** following the regimens in *in vitro* study, clinical studies, and Thai national guideline. None of the regimens were achieved target $C_{trough} \geq 0.7$ mg/L on day 1. **B.** following the proposed regimens, regimen 7 and 8 could achieve target $C_{trough} \geq 0.7$ mg/L on day 1, and maintain $C_{trough} \geq 0.7$ mg/L over 14 day with 10-day course of therapy; OD = once daily, BID = twice daily, TID = three times daily, D = day

4. DISCUSSION

The simulation of HCQ concentration at infected lung tissue is the ideal method for determine the dosage in COVID-19. Despite concentration in lung epithelial lining fluid of HCQ 400 mg once daily or 200 mg three times daily is higher than the maximum EC50 seen in critically ill COVID-19 patients[19], the absence of correlation with clinical outcome and heterogeneity of EC50 values through modelling techniques are limited the dosage determination. Therefore, we use plasma concentration that correlate with virological clearance as PKPD target.

The incapability to achieve the PKPD target of 400 mg daily for 5 days (regimen 1) with the maximum PTA of 13.42% on day 5 in our simulation was consistent

with the absence of increasing virological clearance rate in the study of mild COVID-19 patients, whereas patients' baseline characteristics were comparable between placebo and hydroxychloroquine groups[11]. Similarly, loading dose of HCQ 400 mg twice daily on the first day followed by 200 mg twice daily for 4 days (regimen 2) was insufficient in our simulation (maximum PTA of 29.33% on day 5). This regimen was proposed by using physiologically-based pharmacokinetic models that reached the target free lung trough concentration over half maximal effective concentration (EC_{50}), however the validity was limited due to animal pharmacokinetics data and *in vitro* target[7]. HCQ 200 mg three times daily (regimen 3) showed delayed achievement of PTA (91.49% PTA on day 10). Although virological clearance defined by negative results of SARS-CoV-2 RNA PCR from the nasopharyngeal swab was shown on day 3-6 of therapy in the study of mild COVID-19 patients using regimen 3, the PCR was still positive up to day 6 of therapy in the patients using the same regimen who had older age (mean age; 45.1 vs 58.7 years old) and cancer[9,10]. In addition, Thai national guideline recommends the loading dose of HCQ 600 mg twice daily on the first day followed by 400 mg twice daily for 9 days (regimen 4)[6], however this regimen showed delayed achievement of PTA (92.76% PTA on day 7). The virological clearance outcome of regimen 4 has not yet been evaluated in the clinical study. Even the loading dose of HCQ 1,200 mg once daily for three days followed by 800 mg once daily (regimen 5) could achieve 92.4% PTA on day 7 in our simulation. This regimen showed no increase in virological clearance rate in mild to moderate COVID-19 patients as compared with the standard of care alone, however, the absence of HCQ benefit from delayed treatment cannot be excluded[12].

Some host factors were related to prolonged viral shedding and severity including male sex, and cancer[2, 20-22]. The specific mechanism of differences in duration of infections and risk for complication is unclear. Female sex hormones could suppress the SAR-CoV replication and decrease accumulation of inflammatory monocyte macrophages in the lung of female mice[23]. Immunosuppressive state is possibly the cause of delayed viral clearance in cancer patients[24]. While the host factors related to poor outcome cannot be modified, we were able to optimize HCQ regimens that could maximize the viral load reduction. Moreover, higher levels of cytokine storm indicated by increased levels of proinflammatory cytokines including interleukin (IL)-2, IL-4, IL-6, IL-10, along with tumor necrosis factor (TNF)-α and interferon (IFN)-γ were associated with more severe disease development in COVID-19 patients. Among several proinflammatory cytokines, IL-6 and IL-10 were rapidly increase in more severe group[25]. IL-6 contributes to host defence against infections; however, excessive synthesis of IL-6 leads to an acute severe systemic inflammatory response. HCQ had the dose-dependent reduction of T helper 17 cell-related IL-6 production in autoimmune patients, whereas IFN-α reduction was limited in long term HCQ use for 6 months[26,27]. Therefore, there was a possibility that early increase of HCQ concentration might be benefit by timely reducing overproduction of IL-6 in COVID-19 patients.

Thus, we proposed the HCQ dose regimens of 1,200 mg twice daily on the first day followed by 400 mg twice daily for 9 days (regimen 7) and 800 mg three times daily on the first day followed by 200 mg three times daily for 9 days (regimen 8). These two regimens could achieve the target plasma $C_{trough} \geq 0.7$ mg/L for virological clearance since the first day and thereafter, over 10-day course of HCQ therapy. This is the first study that proposed the high loading dose 2,400 mg in the first day, but each dosage was not exceeded the regimen in published clinical study[12]. Our results were supported by PK properties. HCQ has long plasma half-life (32±9 days)[16], therefore, steady-state plasma concentration cannot be achieved within the treatment course of COVID-19, if the adequate loading dose is not given. In addition, the loading dose should be high enough to compete with the large volume of HCQ distribution (1,390 L) to the peripheral compartments[15], and to rapidly achieve the target C_{trough} in the central compartment. Also, to overcome the large distribution of HCQ to peripheral compartment, increasing dosage frequency is needed to maintain C_{trough} in the central compartment above the target throughout the duration of treatment[28]. For these reasons, our proposed regimen that the loading dose was increased to 2,400 mg per day together with the frequency of 8-hour interval (regimen 7) was the optimal regimen as shown in the PTA analysis (Table 3), while regimen 8, with 12-hour dosing interval was optional for settings with restricted access to COVID-19 patients.

In the context of safety, we also evaluated the probabilities to achieve the toxic level by using simulated C_{max} of 4 mg/L, which all our proposed regimens (regimen 6-8) provided PTA less than 1% over a 10-day course of HCQ therapy. Maximum concentration-related gastrointestinal (GI) adverse events (AEs) might be a concern when our regimen is chosen[29], therefore decreased peak concentration by split-dosing during the treatment course should be applied to reduce the GI AEs. Conversely, retinal toxicity was identified in patients taking HCQ for greater than 5 years[30], this AE would less likely occur during a short period for COVID-19 treatment[12].

The most concerned AEs of HCQ is the QTc prolongation, however the relationship between HCQ concentration and QTc prolongation is currently unknown. The inhibition of the inward rectifier K+ channels is likely to be associated with QTc prolonga-

tion but the half-maximal inhibitory concentration (IC50) of HCQ was not reported in *in vitro* study[31]. In a PK study of 13 critically ill COVID-19 patients, two patients had to discontinue HCQ due to QTc prolongation and they had HCQ whole blood concentration of 0.03 g/L and 1.74 mg/L. Risk factors associated with QTc prolongation were not reported in this study, but QTc prolongation from critical illness cannot be excluded[32]. Furthermore, the randomized controlled trial of hospitalized COVID-19 patients received HCQ 2,000 mg in the first day (800 mg every 6 hours for 2 doses then 400 mg at 12 hours after the initial dose) followed by 400 mg twice daily for 9 days did not show any excess in ventricular tachycardia or ventricular fibrillation compared with usual care[33].

Many risk factors contributing to QTc prolongation have been reported, including female sex, structural heart diseases, electrolyte disturbances, hepatic/renal failure, baseline QTc > 450 milliseconds, concomitant QTc prolonging medications and ICU status at the time of treatment[34,35]. Due to limited data of high dose HCQ usage in patients with risk factor of QT prolongation, our regimens may not be recommended in patients with risk factor of QT prolongation, or balancing risk and benefit before use has to be considered.

Centers for Disease Control and Prevention (CDC) and National institute of health (NIH) are not recommended hydroxychloroquine for treating COVID-19 based on recent clinical studies that showed no additional benefit on mortality reduction or improving clinical COVID status to usual care[33,36,37]. However, underdosing lead to a potential lack of benefit cannot be exclude. Even the highest dose in randomized controlled trial, HCQ 2,000 mg in first day (800 mg every 6 hours for 2 doses then 400 mg at 12 hours after the initial dose) followed by 400 mg twice daily for 9 days showed delayed achievement of PTA in our simulation. Therefore, we proposed dose regimens to further investigate in clinical studies, principally the plasma concentration-effect relationship.

Our study has some limitations. First, we simulated the plasma HCQ concentration using population PK parameters derived from both healthy and malaria-infected patients, which may be altered in severe COVID-19 patients. For example, GI absorption was decreased due to shunting of blood to vital organs, distribution was increased due to inflammation state, and elimination was decreased in hepatic or renal failure patients[38]. Second, we used plasma concentration that achieves virological clearance in nasopharyngeal swab as PKPD target, which may not be completely correlated with clinical improvement in COVID-19 patients. However, it is generally accepted that viral clearance in nasopharyngeal swab is a desirable marker for clinical improvement and transmission prevention[39].

5. CONCLUSIONS

The two optimal HCQ regimens that may help for early viral clearance in COVID-19 patients were HCQ 800 mg three times daily on the first day followed by 200 mg three times daily for 9 days, and HCQ 1,200 mg twice daily on the first day followed by 400 mg twice daily for 9 days. Further clinical study is needed to ensure efficacy and safety of these regimens.

6. ACKNOWLEDGEMENT

We would like to thank Phinnaphit Saengpao for her help throughout the study. My sincere thanks also go to my beloved mother and wife for their encouragement throughout the research period.

Conflict of interest

The authors declared no competing interest for this work.

Funding

This study received no external funding.

Ethics approval

This study was approved by the Institutional Review Board of Faculty of Dentistry/Faculty of Pharmacy, Mahidol University (COE.No.MU-DT/PY-IRB 2020/ 014.2005).

Article info:

Received January 4 2021
Received in revised form April 27, 2021
Accepted May 13, 2021

Authors' contributions

PM, SL conceived and designed the study. SL, TP, ST performed the research. PM, SL analysed and interpreted the data. SL, PD wrote the manuscript. PM, SK, PD, SC revised manuscript and all of the authors approved the final manuscript.

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