How Could In Vitro Antiviral Activity Be Applied to Optimize the Dosing Regimens of Candidates for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)?

To the Editor—Recently, Yao et al reported an optimized dosing regimen design of hydroxychloroquine (HCQ) for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on model and simulation (M&S) [1]. The authors integrated the in vitro antiviral activity and the target tissue (lung) concentration of HCQ using physiologically based pharmacokinetic (PBPK) model. This study provided a novel strategy for optimization of HCQ dose regimen and potentially could be applied for other antivirus drug candidates. However, the recommended dosing regimen proposed by Yao et al cannot reach the effective antiviral concentration in vivo, even assuming HCQ is an effective candidate for anti-SARS-CoV-2, because the target tissue (lung) concentration was overestimated and then mismatched the in vitro experiment result. We think that the application of PBPK modeling and simulation to optimize clinical study must rely on rigorous pharmacokinetic mechanism and reasonable assumption.

THE TARGET TISSUE (LUNG) CONCENTRATION OF HCQ WAS OVERESTIMATED AND MISMATCHED THE IN VITRO ACTIVITY (EC50)

To evaluate dosing regimens, Yao et al used the ratio of predicted free lung trough concentration (C_{\text{trough, lung}}) of HCQ to its concentrations in vitro that led to 50% of the maximum antiviral activity (half-maximal effective concentration, EC50 value) as pharmacodynamics index, that is, R_{\text{LTEC}} = \frac{C_{\text{trough, lung}}}{\text{EC50}} [1]. Here the EC50 value referred to the concentration in the cell culture medium, and C_{\text{trough, lung}} was simulated based on plasma concentration adjusted by the lung-to-plasma tissue partition coefficient (K_p) and unbound fraction (f_{\text{u, plasma}}) in plasma (C_{\text{trough, lung}} = C_{\text{plasma}} \times K_p \times f_{\text{u, plasma}}).

Remarkably, the simulated lung concentration (C_{\text{trough, lung}}) should be the free lung extracellular concentration (Figure 1) because the EC50 value is the extracellular concentration (the cell culture medium). However, the K_p values used by Yao et al was much lower than the extrapolated rat-to-human value (21–169). Remarkably, the simulated lung concentration was overestimated (CTRough, lung/EC50, value 21–169). Recently, an article from the US Food and Drug Administration Office of Clinical Pharmacology (Fan et al) proposed that the free lung extracellular concentration may be approximately equal to the plasma free concentration [5] (Figure 1). We think the predicted lung concentration must correctly match the EC50 value in vitro when bridging of antiviral activity in vitro and the concentration in vivo. Therefore, the reevaluated R_{\text{LTEC}} value (0.017–0.34) based on the new free lung extracellular trough concentrations (C_{\text{plasma}} \times K_p \times f_{\text{u, plasma}}) rather than the overestimated “free lung trough concentrations” (C_{\text{plasma}} \times K_p \times f_{\text{u, plasma}}) predicted by Yao et al was much lower than the estimated R_{\text{LTEC}} value by Yao et al (21–169) [1, 5]. This means that the highest dosage regimen (D1 800 mg + 400 mg; D2–D10 400 mg QD) based on this higher R_{\text{LTEC}} value proposed by Yao et al cannot reach the effective antiviral concentration even if HCQ is an effective anti-SARS-CoV-2 drug candidate.

THE IN VITRO ACTIVITY (EC50) WAS SIGNIFICANTLY AFFECTED BY EXPERIMENTAL FACTORS

The evaluation of antiviral activity (EC50 value) in vitro is significantly affected by experimental factors, including the experimental methods and conditions. According to Yao et al, the anti-SARS-CoV-2 activities of HCQ were characterized using EC50 value, which were determined by quantification of viral RNA in the cell supernatant at 0.01 multiplicities of infection (MOIs) and represented the extracellular concentration of HCQ [1]. The results showed that the EC50 value of HCQ was 6.14 μM and 0.72 μM in African green monkey kidney cells (Vero cells) at 24 and 48 hours postinfection, respectively [1].

However, there was a significant difference in EC50 value of HCQ under different experimental conditions [7, 8]. For example, the EC50 value of HCQ ranged from 1.13 μM to 17.31 μM at different MOIs (0.02–0.8) [7, 8]. And there was no antiviral activity observed in a model of reconstituted human airway epithelium [9]. In addition, Liu et al found that the EC50 value of HCQ was 4.51 μM under the same experimental conditions (0.01 MOIs, 48 hours postinfection, Vero cells), which was about 6-fold higher than that of Yao et al [7]. In brief, the determination of EC50 value in vitro can be significantly influenced by many experimental factors, and there is a marked difference between labs even if the method is the same. Because the parameter R_{\text{LTEC}} (C_{\text{trough, lung}}/\text{EC50}) is the key pharmacodynamics index in evaluation of dosing regimen for antiviral drugs based on PBPK modeling and in vitro antiviral activity, the factors affecting the EC50 analysis should be taken into account.
account. In addition, the measurement of antiviral activity (EC\textsubscript{50} value) in vitro requires rigorous methods, and the EC\textsubscript{50} value alone is not sufficient to judge a drug’s in vivo antiviral activity [10].

**IN CONCLUSION**

- PBPK model is a novel strategy to optimize the dosing regimens by using antiviral activity in vitro; however, the development of this model must be based on reasonable assumption.
- The predicted target tissue (lung) concentration must correctly match the EC50 value in vitro.

**Notes**

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