Table 1. Hydroxychloroquine Simulation Results

Study	Parameter	Day 1	3	5	8	10
Yao et al, [1] regimen "F"	Dose every 12 hours, μM	922	461	461	0	0
	C_{min}, lung, free, μM	14 (C_{max})	39	57	64	61
	C_{min}, lung, free/EC_{50} calculated	2.3[a]	54	79	88	85
	C_{min}, lung, free/EC_{50} reported (Table 1)	21[a]	38.9	85.4	NA	83.3
Wolowich/Collins [3] regimen "F"	C_{min}, lung tissue, free, μM	4.6 (C_{max})	12.7	22	10.5	7.2
	C_{min}, lung tissue, free/EC_{50}	1.0[a]	17.6	30.6	14.6	10
	C_{min}, lung lysosomes, μM	995	26 820	30 433	23 578	20 604
	Amount of HCQ in lung that is in the lung lysosomes, %	98	99.9	99.8	99.9	99.9

Abbreviations: C_{max}, maximum concentration; C_{min}, minimum concentration; EC_{50}, concentration that kills 50% of virus; HCQ, hydroxychloroquine; NA, not available.
[a]EC_{50} = 6.14 μM all other timepoints (3,5,8,10 h) EC_{50} = 0.72 μM.

concentrations as these data are not presented in tabular form in the manuscript. Yao reported the predicted lung-to-plasma ratio of HCQ was 400 to 1; using a blood-to-plasma ratio of 7.2, this is a lung-to-blood ratio of 56 to 1. This figure is misleading as our simulation demonstrates that 98% of the drug in the lung is sequestered in the lung lysosomes after the first dose of the regimen (Table 1). Our simulation also predicts lysosomal concentrations in the lung to be >20 000 μM for the majority of the 5-day dosage regimen and the 5-day postregimen period. The lack of lysosomal binding in a model used to predict the concentration profile of a drug known to be highly lysomotropic overestimates drug in the lung tissue, as a significant amount of the drug is sequestered in the lysosomes. Whether the drug in the lysosomes retains antiviral activity is unknown.

We also examined the model used by Yao et al available on the Simcyp website [2]. Several discrepancies were noted. Bioavailability (F) in the Tett et al [4] study used to calibrate the model was 0.75; Yao used F = 1 in the simulations. The Tett et al study absorption rate constant ka was 0.5 h-1; Yao used 0.8 h-1. Yao et al admits the use of a "high" lung-to-plasma tissue partition coefficient (Kp) derived from animal studies. The actual value used was 108 with a scalar of 2.45, meaning the Kp was 265. As has been documented several times in the literature, Kp derived from animal tissue homogenate should not be used in PTA analysis [5–8]. They also employed an additional organ with a Kp of 547; when the scalar of 2.45 is applied, the Kp is 1340. The net result of these discrepancies will underestimate drug in the blood, overestimate drug in the lungs, and moreover place the drug in the lung tissue and not the lung lysosomes. These findings suggest that the model predictions published by Yao et al are suspect and could mislead practitioners to use the drug in COVID patients with little evidence of efficacy.

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Reply to Wolowich and Kwon

To the Editor—We thank Wolowich and Kwon for their letter commenting on our earlier publication [1], and appreciate the opportunity to respond.

The authors stated that "Yao concludes a dosage regimen of oral HCQ [hydroxychloroquine] provides sufficient lung exposure to exceed the viral half-maximal effective concentration (EC_{50}) and thus the regimen should be effective in treating COVID-19 patients. This conclusion is not warranted from the data presented," and further that "these findings … could mislead practitioners to use the drug in COVID-19 [coronavirus disease 2019] patients with little evidence of efficacy." This significantly misinterpreted our publication. The only conclusion we made was that "HCQ was found to be more potent than CQ [chloroquine] to inhibit SARS-CoV-2 [severe acute respiratory syndrome coronavirus 2] in vitro" [1]. Although one can employ modeling and pharmacology concepts to predict the likelihood of clinical efficacy from in vitro data, given the inherent limitations of any modeling approach and assumptions being made, in vitro efficacy can only be ultimately confirmed through

clinical trials. To this end, any modeling analysis has to be fit for purpose. In our article [1], the purpose of physiologically based pharmacokinetic (PBPK) modeling was to provide timely support on dosing decisions for our clinicians, who eventually used safe doses of CQ and HCQ that were approved for other indications to treat patients with COVID-19 in Wuhan, China. Specifically, we tried to use PBPK modeling first to understand why CQ was found to have clinical antiviral effects [2], and then to determine the relative potency between HCQ and CQ based on in vitro EC_{50} values and respective drug models. To date, the clinical antiviral activities of CQ and HCQ remain to be confirmed [3, 4].

Tissue-to-plasma partition coefficient (Kp) is a critical bridging parameter to estimate tissue concentration [5]. As we clearly recognized in our Discussion, we made several assumptions in our analysis that require further validation and refinement. We used a perfusion limited tissue distribution model to mimic the time-dependent lung Kp characteristics [1], where we assumed the same lung Kp characteristics of HCQ as that of CQ (11–547 from 1 hour to 168 hours postdosing) [6], which was similar to lung Kp value in mice (average, 29×7.2 from 6 hours to 72 hours postdosing) reported by Chhonker et al [7] and our newly generated monkey data (~200 at 24 hours postdosing; Liu et al, unpublished data). We also benchmarked CQ exposure under 500 mg twice daily for 10 days, which first showed antiviral effect in Chinese patients [2], and calculated a lung tissue to plasma concentration (R_{LTEC}) of HCQ under regimen "F" to be greater than CQ. Wolowich and Kwon stated that they used a "slightly modified version of an HCQ model published by Collins et al" [8], and we respectfully disagree. The model by Collins et al was developed in different software with different model assumptions, where we did not see how to simulate lung tissue concentration. As we are not able to see what Wolowich and Kwon simulated, we hereby point out the following assumptions/observations reported in Collins et al's article [8] that warrant further discussion:

1. Kp was set as a constant rather than a time-dependent function, whereas animal studies [6], as well as the unpublished Liu et al study, suggest time-dependent accumulation of both CQ and HCQ; we tried to capture the time-dependent drug accumulation by using an additional organ in Simcyp, and acknowledged the limitation of this parameterization in our Discussion. We and others are updating these models (Cui et al [9]; Rowland Yeo et al [10]; Zhang et al, unpublished data).
2. The simulated half-life (68–77 hours; see Table 4 of Collins et al [8]) appears to be much shorter than that observed in clinical studies [11, 12] (~40 days) and our article's simulation results (~20 days) [1].
3. Although Collins et al simulated HCQ concentrations in human lysosomes, these concentrations have not been validated with any nonclinical or clinical data, and HCQ could also be accumulated in other acid cell organs, such as endosome or golgi [13, 14], which could significantly affect lung tissue concentration simulated by Wolowich and Kwon if they just assume HCQ was accumulated in lysosome. Actually, pH increase in acid cell organs, led by HCQ accumulation in these cell organs, was suggested to be a key mechanism to inhibit SARS-CoV-2, although it was not confirmed [15].

We would also like to clarify issues brought up by Wolowich and Kwon, who apparently misunderstood our analysis [1] and the use of Simcyp software:

1. f_a is fraction absorbed in intestine, specifically fraction of drug from gut lumen to enterocytes, rather than bioavailability (F). The simulated F, which actually equals $f_a \times f_g \times f_h$ (f_g and f_h being fractions of drug escaping metabolism within enterocytes and liver first-pass metabolism, respectively) was 0.8, and reported F in humans was 0.75 [11, 12].
2. K_a of 0.5 $hour^{-1}$ of k_a mentioned by Wolowich and Kwon was not found in Tett et al [11], whereas other studies [12, 16] reported 0.194 or 1.15 $hour^{-1}$ in humans, which informed the value of 0.8 $hour^{-1}$ in our article [1].
3. Kp scaler in our PBPK model was not applied to lung tissue because we set lung as an additional organ, which allowed us to mimic the time-dependent tissue distribution profiles (see previous paragraph).
4. We decided not to simulate lysosomal concentration in that we could not validate the lysosome concentration using in vivo data, and again the Simcyp lung model has a different model structure than that reported by Collins et al.

In conclusion, we used PBPK models with the best knowledge available to timely support safe use of CQ and HCQ by our clinicians to treat COVID-19 patients safely in Wuhan and Nanchang City. We declared our assumptions of modeling and acknowledged limitations. Soon after publication, we contributed to medical research by uploading the raw model files to the Simcyp repository so that others can readily apply them [10, 17].

Notes

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Potential conflicts of interest. D. L. has a patent pending for antimicrobial infection pharmaceutical composition and its application. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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SARS-CoV-2-Specific Antibody Detection in Healthcare Workers in a UK Maternity Hospital: Correlation With SARS-CoV-2 RT-PCR Results

To the Editor—During the ongoing coronavirus 2019 (COVID-19) pandemic, staff shortages resulting from illness, self-isolation, and redeployment have been a major challenge. Universal healthcare worker (HCW) testing is potentially useful in ameliorating workforce depletion and reducing asymptomatic spread of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Nasopharyngeal swab reverse transcriptase-polymerase chain reaction (RT-PCR) can diagnose only current or recent infection; testing for antibody responses against SARS-CoV-2 could enhance the ability to expedite reinstatement of services, while ensuring patient and staff safety. Tests are now available for immunoglobulin G IgG against the SARS-CoV-2 nucleocapsid protein; the Abbott SARS-CoV-2 IgG enzyme-linked immunosorbent assay is reported to have high specificity (99.9%) and sensitivity (96.9%) [1]. In London, England, HCW at the Portland Hospital for Women and Children are routinely swabbed for SARS-CoV-2 as part of hospital surveillance policy and asked to self-isolate if infected. Between May 15 and 28, 2020, 190 HCWs who had previously had a nasopharyngeal swab for SARS-CoV-2 were screened for SARS-CoV-2 IgG antibodies. Informed consent included the acknowledgment that a positive result should not be considered an "immunity certificate."

SARS-CoV-2 IgG antibodies were detected in 41 (22%) HCWs, including all 25 (19 [76%] symptomatic, 6 [24%] asymptomatic) previously testing positive for SARS-CoV-2 on nasopharyngeal swab RT-PCR. At the same time, 16/165 (10%) HCWs who tested negative for SARS-CoV-2 on nasopharyngeal swab, of whom 2 (12.5%) had reported COVID-19-like symptoms, were positive for SARS-CoV-2 IgG. Of those positive for IgG, 39% had previously tested negative on nasopharyngeal swab (Figure 1). Risk factors associated with an increased risk of severe COVID-19 are included in the Figure 1.

We previously reported that 32% of HCWs testing positive for SARS-CoV-2 on nasopharyngeal swab were asymptomatic at the time [2]. Symptomatic and asymptomatic SARS-CoV-2-positive adults have similar viral loads and infectious virus isolation [3]. Our finding that both of these groups developed SARS-CoV-2 IgG antibodies is reassuring.

Of those testing positive for SARS-CoV-2 IgG, 39% had an earlier negative nasopharyngeal swab. Possible explanations are that either infection occurred at an interval before or after the swab test, or the swab RT-PCR gave a false-negative result (resulting from poor swabbing technique, suboptimal storage conditions, delay in testing, or poor sensitivity of nasopharyngeal swabs, reported to be as low as 70%) [4].

The overall prevalence of SARS-CoV-2 IgG (22%) among HCWs was higher than in the general population in London (17%) or across the United Kingdom (5%) [5]. Both symptomatic and asymptomatic infections were associated with SARS-CoV-2 IgG antibodies, as were 10% of HCWs with negative nasopharyngeal swabs, despite the majority remaining