Chloroquine and hydroxychloroquine pharmacology relevant to COVID19 prevention and treatment

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Abstract

Enormous clinical experience with chloroquine and hydroxychloroquine has been gained over the past 60 years in the treatment of malaria, amoebic liver abscess, and several rheumatological conditions. COVID19 represents a new potential indication, although there is no convincing evidence at this time of significant efficacy. Dosing based on previous therapeutic guidelines has therefore to be adapted, based on the pharmacokinetic properties of both drugs and their likely pharmacodynamic effects, to increase the chance of providing benefit in this potentially lethal infection. In general, chloroquine and hydroxychloroquine are safe and they are reasonably well tolerated, although these drugs are dangerous when overdosed and parenteral administration needs careful control. This is because of their unusual pharmacokinetic properties. Chloroquine and hydroxychloroquine have enormous apparent volumes of distribution (chloroquine $>$ hydroxychloroquine) and they are both eliminated very slowly from the body (terminal elimination half-lives $>$ 1 month). Thus, the free plasma concentrations which drive potentially serious adverse reactions (hypotension, conduction disturbances, delayed ventricular repolarization, and neurotoxicity) are determined largely by distribution processes. Hydroxychloroquine has been shown to be slightly safer than chloroquine in preclinical testing and is considered better tolerated over the long term. Considering the blood concentrations of both drugs that have been documented in the acute treatment of malaria (over 3 days), the chronic treatment of rheumatological conditions (taken over years), the enormous clinical experience in these conditions, the toxicokinetics in self-poisoning, and the potentially weak in vivo activity against the SARS-CoV-2 virus, current clinical trials are using the highest doses of these drugs that are considered to be reasonably safe. Using lower doses risks failing to identify any benefit in a potentially lethal infection. Large, well conducted randomised clinical trials with appropriate monitoring are required to determine if these drugs have preventive or treatment efficacy and acceptable safety. Current recommendations for their use outside of clinical trials are not justified at this time.

1 Background

Chloroquine [7-chloro-4-[[4-(diethylamino)-1-methylbutyl]amino] quinoline is a 4-aminoquinoline compound discovered in Germany in 1934 as part of a research programme to develop new antimalarial drugs, although it was not evaluated fully until the end of the Second World War \cite{1} \cite{2}. Hydroxychloroquine, in which one of the ethyl groups in the alkyl side chain is hydroxylated, was synthesized in 1946 (Figure \ref{fig:1}). Early clinical pharmacology assessments in the USA characterized the safety, tolerability, and antimalarial efficacy of chloroquine and it was selected from a
Figure 1: Chemical structure of chloroquine (C_{18}H_{26}ClN_{3}; MW: 319.872 g/mol) and hydroxychloroquine (C_{18}H_{26}ClN_{3}O; MW: 335.872 g/mol).

Table 1: Comparison of animal toxicity of chloroquine and hydroxychloroquine by McChesney et al. (1983) [8].

<table>
<thead>
<tr>
<th>Species and Route of Administration</th>
<th>Acute LD_{50}, mg/base/kg</th>
<th>Tolerated Dose, mg/base/kg per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, IV</td>
<td>25 ± 2^{T}</td>
<td>45 ± 2^{T}</td>
</tr>
<tr>
<td>Mouse, IP</td>
<td>79, 66-78</td>
<td>182^{T}</td>
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<tr>
<td>Mouse, oral</td>
<td>367 ± 50, 1,000^{T}</td>
<td>1,880 ± 133^{T}</td>
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<tr>
<td>Rat, oral</td>
<td>&lt;3/20, &lt;600^{T}</td>
<td>400 = LD_{50}^{T}</td>
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<tr>
<td>Rat, oral</td>
<td>608 ± 196</td>
<td>&gt;50, &lt;100^{T}</td>
</tr>
<tr>
<td>Dog, IM</td>
<td>6-8^{T}, &lt;8^{T}</td>
<td>&gt;25^{T}</td>
</tr>
<tr>
<td>Dog, oral</td>
<td>&gt;12.5, &lt;50^{T}</td>
<td>&gt;12, &lt;20^{T}</td>
</tr>
<tr>
<td>Rabbit, IV</td>
<td>&gt;12.5, &lt;19.5^{T}</td>
<td>&gt;25, &lt;50^{T}</td>
</tr>
<tr>
<td>Monkey, oral</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

TABLE I Some Important Parameters of Animal Toxicity of Chloroquine (CQ) and of Hydroxychloroquine (HCQ)

Industrial production peaked in 2004 when, in the last quarter of the year, China alone reported production of over 400 tonnes [4]. Thus, well over 5 billion treatments have been dispensed worldwide, and chloroquine can claim to be among the drugs to which humans have been most exposed. Chloroquine has been used both in the prevention and treatment of malaria.

Today, although *Plasmodium falciparum* is resistant to chloroquine everywhere except in Haiti and Central America north of the Panama Canal, chloroquine remains a first line treatment for non-falciparum malaria [5]. Also, chloroquine was, and it still is, used to prevent malaria in pregnancy [5–7]. In the 1950s chloroquine was even added in large quantities to salt in some regions to provide mass antimalarial prophylaxis. At the same time the other potential benefits from this drug class were being explored. Chloroquine proved to be effective in the treatment of amoebic liver abscesses and to possess important anti-inflammatory properties.

Hydroxychloroquine was shown to have some safety advantages in experimental animals [8], and although it is as good as chloroquine as an antimalarial, it was developed more for its use in rheumatological conditions [9]. Hydroxychloroquine is generally considered to be slightly safer than chloroquine (Table 1 shows animal data) although the evidence to support this in man is not strong. The treatment of malaria required a short course regimen (25mg base/kg total dose-up to 50mg/kg over 2 or 3 days whereas high total doses (10mg base/kg daily for two days followed by 5mg base/kg daily for 2-3 weeks) were used for the treatment of hepatic amoebiasis. This review focuses on the clinical pharmacology of these two 4-aminoquinolines in relation to potential preventive and treatment use in COVID19. It does not review in detail the evidence for antiviral activity.
2 Chemistry

Chloroquine and hydroxychloroquine are dispensed as racemic mixtures. The enantiomers have different pharmacokinetic (see below) and pharmacodynamic properties \[10, 11\]. Hydroxychloroquine is more hydrophilic than chloroquine.

3 Clinical Pharmacokinetics

Even in the 1940s, when drug measurement was in its infancy, it was clear that the 4-aminoquinolines had unusual pharmacokinetic properties \[2\]. Absorption after oral administration was rapid and generally reliable, but the total apparent volume of distribution was enormous (>100 L/kg) reflecting extensive tissue binding \[12, 30\]. Hydroxychloroquine appears to have a smaller total apparent volume of distribution but otherwise similar absorption, distribution and elimination kinetics \[8, 25, 28, 31, 41\]. Estimates for the terminal elimination half-life lengthened as the less sensitive spectrophotometric assays were replaced by high performance liquid chromatography methods (i.e. with increasing sensitivity a greater proportion of the terminal phase could be characterised). The true terminal elimination half-life of chloroquine approximates 1-2 months \[14, 15\]. The quoted average values for terminal phase elimination half-lives for chloroquine \(~38 \text{ days} \) and hydroxychloroquine \(~54 \text{ days}\) may not represent significant differences \[14, 15, 42\]. Thus, the initial plasma or whole blood concentration profile in the treatment of acute illness is determined mainly by distribution processes and not by elimination \[14, 17, 19, 21\]. This is critical to understanding the relationship between dosing and concentration profiles, and associated risks with short course treatments. In the treatment of malaria (or COVID19) the initial (loading) doses are designed to “fill” the body so that concentrations that would take weeks to achieve without a loading dose, are achieved as soon and as safely as possible. In chronic dosing maintenance doses are then much lower.

The dosing and spacing of the loading dose aims to provide sufficient time for chloroquine or hydroxychloroquine to diffuse out from the small central “compartment” and thereby avoid accumulation to transient very high concentrations that are potentially toxic (Figure 2) \[17\]. Presumably free chloroquine equilibrates at different rates with different tissues and cellular components but the vascular smooth muscle and cardiac muscle seem to be in rapid equilibrium such that haemodynamic and cardiac electrophysiological changes occur almost synchronously with blood concentrations so there is little hysteresis in the cardiovascular concentration-effect relationship \[17\].

The plasma or blood concentration profile of chloroquine or hydroxychloroquine in acute treatment is determined primarily by distribution not elimination.

Orally administered chloroquine is well absorbed, even in unconscious patients \[13, 15, 18, 21\]. It is very rapidly absorbed following subcutaneous (or intramuscular injection). Absorption may outpace distribution, and with subcutaneous or intramuscular doses of 5mg base/kg or more transiently toxic concentrations may occur \[16, 21\]. To circumvent this, intravenous chloroquine is administered by constant-rate infusion, and subcutaneous or intramuscular chloroquine is given in small (2.5-3.5mg base/kg) frequent injections \[19, 21\]. The principal metabolite of chloroquine, desethylchloroquine, has approximately equivalent antimalarial and other biological activities \[11, 43, 44\]. Measurement of chloroquine and hydroxychloroquine in blood is complicated by extensive binding of the drugs within leukocytes and platelets, and to a lesser extent erythrocytes \[15, 46\]. As a result plasma levels (with adequate centrifugation at 2000g for >10 minutes) are half those in serum, and approximately four times lower than whole blood for hydroxychloroquine, and five times lower for chloroquine, although there is substantial variability in the published literature, see Table 2 \[17\]. For these reasons whole blood is the preferred matrix for pharmacokinetic studies. Early spectrophotometric assays used before the 1990s lacked specificity in distinguishing the desethylated metabolites and were relatively insensitive \[45\]. Most recent studies have used HPLC with UV detection.
Chloroquine or hydroxychloroquine whole blood concentrations

Figure 2: Example of a general concentration-time profile for chloroquine or hydroxychloroquine.

Table 2: Chloroquine and hydroxychloroquine blood to plasma ratios, reproduced from Table 3 in Megarbane et al. 2010 [47].
4 Enantiomer binding and elimination kinetics

Using HPLC assays the estimated mean terminal elimination half-life was longer for (R)-chloroquine (12.3 days) than for (S)-chloroquine (9.8 days) [49, 50]. The estimated mean total body clearance was lower and distribution volume was smaller for the (R)-enantiomer (14.2L/h and 720L, respectively) than for the (S)-enantiomer (8.16L/h and 3410L, respectively). For hydroxychloroquine, in 8 patients on chronic dosing the blood concentration of (R)-hydroxychloroquine also exceeded that of the (S)-enantiomer, with mean (R)/(S) ratio of 2.2 (range 1.6-2.9). The mean enantiomer blood concentration ratio (R)/(S) for the metabolite desethylhydroxychloroquine was 0.45 (range 0.34-0.58) and 0.56 (range 0.35-0.86) for desethylchloroquine suggesting stereoselective metabolism of hydroxychloroquine. (S)-hydroxychloroquine mean (SD) renal clearance from blood was 41±11 ml/min, approximately twice that of (R)-hydroxychloroquine [51, 52]. Protein binding was also different for the chloroquine stereoisomers, with opposite preferential binding to human albumin and the “acute phase protein” alpha 1-acid glycoprotein. Total human plasma protein binding was 67% for (S)-chloroquine and 43% for the (R)-enantiomer [46]. The plasma protein binding estimates for hydroxychloroquine are very similar [10]. The (S)-enantiomer of hydroxychloroquine was 64% bound in plasma, while (R)-hydroxychloroquine was 37% bound. The S enantiomers showed relatively greater binding to albumin and lower binding to alpha 1-acid glycoprotein. Thus, in contrast to the avid tissue binding, chloroquine and hydroxychloroquine are not highly bound to plasma proteins. Both drugs are metabolized slowly. Renal elimination accounts for 20-55% of total clearance. Using spectrophotometric assays McChesney et al. estimated that 55% of an oral chloroquine dose was eliminated in the urine with 70% as unchanged drug, 23% as desethylchloroquine, 1-2% as bidesethylchloroquine, and the remainder as other metabolites [33]. Hydroxychloroquine is also desethylated and the hydroxyl group is also removed so that desethylchloroquine and chloroquine are formed as well (Figure 3). N-desethylation is mediated by CYP 2D6, 3A4, 3A5, and 2C8 isoforms [53]. CYP2D6 polymorphisms do affect the steady state ratio of parent drug to active metabolite [30]. Clinically significant pharmacokinetic drug-drug interactions with hydroxychloroquine and chloroquine have not been reported. As described above, drug metabolism and clearance have little effect on the plasma or whole blood concentration profiles in the first few days of treatment, but they are relevant to chronic dosing. Accumulation occurs slowly with repeated dosing (as in rheumatological conditions or continuous chemoprophylaxis) because of the very long terminal elimination half-life (e.g. Figure 4) and reaches higher levels in patients with renal failure [33, 41], although the steady state levels are often not as high as would be predicted from renal clearance estimates in healthy subjects. It is possible that non-renal clearance increases as glomerular filtration falls.

5 Antiviral and antimalarial activities

The 4-aminoquinolines inhibit the pH-dependent steps of replication of a broad range of viruses (including flaviviruses, retroviruses, and coronaviruses) [30, 54–58]. The exact mechanism of antiviral action is unclear as these drugs may interfere with nearly every step of cellular infection and replication, i.e. viral fusion, viral penetration, nucleic acid replication, viral protein glycosylation, virus assembly, new virus particle transport, and virus release [30, 54, 55]. Activities (EC50s) against the SARS-CoV-2 virus are in the low micromolar range which represents the upper end of the free plasma concentration range encountered in clinical practice [30, 55, 58]. Whether these estimates, derived usually from Vero cell cultures are representative of the respiratory epithelium is not known. The antimalarial mode of action of the quinoline antimalarials has been a source of controversy for years [5]. These drugs are weak bases, and they concentrate in the acid food vacuole of the parasite, but this in itself does not explain their antimalarial activity. Chloroquine intercalates DNA, but only at concentrations much higher (1-2µM) than required to kill parasites (10-20nM). Chloroquine binds to ferriprotoporphyrin IX, a product of haemoglobin degradation, and thereby chemically inhibits haem dimerization. This is an essential defence mechanism for the malaria parasite to detoxify haem, and inhibition of this process provides a plausible explanation for the selective antimalarial action of these drugs [59]. Chloroquine also competitively inhibits glutathione mediated haem degradation, another parasite detoxification pathway [60].
6 Formulations

Chloroquine has been formulated as sulphate, phosphate, and hydrochloride salts, and is prescribed for malaria in weights of base content. Various liquid formulations are available for paediatric use. Chloroquine can be given by intravenous infusion, intramuscular or subcutaneous injection, orally, or by suppository [5]. Hydroxychloroquine has been made in a parenteral formulation but the usual form is a tablet of the sulphate salt.

There are different salts, each with a different base equivalent. This has led to confusion and sometimes mistakes in dosing. Malaria treatment is usually recommended in terms of base. Chloroquine diphosphate 250 mg salt contains 155 mg base. Hydroxychloroquine sulphate 200 mg salt contains 155 mg base.

7 Toxicity

Chloroquine and hydroxychloroquine are generally well tolerated. The most common adverse reactions reported are dyspepsia, nausea, occasionally vomiting, visual disturbances (particularly transient accommodation difficulties), and headache [61–65]. The gastrointestinal adverse effects can often be lessened by taking chloroquine with food. Orthostatic hypotension may be accentuated in febrile patients [66].

The main concern with high doses is cardiovascular toxicity [67]. Parenteral chloroquine causes hypotension if administered too rapidly or when a large dose (>3.5 mg base/kg) is given by intramuscular or subcutaneous injection [17, 21]. In addition, chloroquine and hydroxychloroquine (and the structurally related 4-aminoquinoline amodiaquine and the bisquinoline piperaquine) block several different cation channels [68–71]. In voltage-clamped cat ventricular myocytes, chloroquine blocked several inward and outward membrane currents. The order of potency was: inward rectifying potassium current (IK1), rapid delayed rectifying potassium current (IKr), sodium current (INa), and L-type calcium current (ICa-L) [69]. Chloroquine blocked the rapid component of the delayed rectifying outward current, IKr, but not the slow component, IKs. This explains the prolonged QT interval, impaired ventricular conduction (resulting in ECG QRS widening), and increased automaticity. Chloroquine and hydroxychloroquine also block the hyperpolarization-activated funny current (If) (as does ivabradine). This plays a major role in the sino-atrial node.
Figure 4: Measured whole blood concentration data showing the long terminal elimination of chloroquine (top) and hydroxychloroquine (bottom). The top panel shows data from [15]; the bottom panel shows data from [31]. The inset in the bottom panel shows the first 100 hours after drug administration.
pacemaker and thereby may cause bradycardia \[71\]. Blockade of the inwardly rectifying Kir2.1 potassium (hERG) channel, which delays ventricular repolarization and thereby prolongs the ECG QT interval, has been a focus of concern \[72, 74\]. This is a risk factor for polymorphic ventricular tachycardia (TdP: torsade de pointes), although there is much debate about the determinants of the risk relationship \[73\], and the potential ameliorating effect of multichannel blockade \[76\]. While chloroquine and hydroxychloroquine do prolong the electrocardiograph J to T-peak interval, and are potentially “torsadogenic”, the extent to which the risk of TdP is increased with chloroquine or hydroxychloroquine alone is unclear. TdP has been reported in chronic dosing in systemic lupus erythematosus (SLE) \[74\], where myocarditis may be a confounder, and in overdose, but there is very little evidence for significant risk in acute treatment with the doses that have been used in malaria or rheumatological conditions \[74, 85\]. Sudden unexplained death has not been associated with chloroquine previously despite wide variation in dosing \[74\], although in malaria treatment there have not been very large prospective studies to assess this definitively despite the enormous usage. In the extensive malaria prophylaxis experience there have also been no established associations with sudden unexplained death. Recent prospective studies with data from 200,000 patients with the related bisquinoline antimalarial compound piperaquine, which has very similar hERG blocking properties, found no increased risk of TdP after standard treatment \[77\]. In contrast, chloroquine clearly does have beneficial anti-arrhythmic properties which are under recognised \[78, 82\]. This is particularly useful in SLE where arrhythmias are common and patients on hydroxychloroquine are relatively protected \[83\].

Disease-toxicity interactions may also be relevant. Malaria and malarial fever have independent effects on the QT interval and heart rate \[84\], although the heart is relatively spared even in severe malaria \[85\]. There is evidence of cardiac injury in COVID19 \[86\], with myocarditis proposed as a potential cause, but the mechanism for these changes is as yet unclear. Whether patients receiving high dose chloroquine or hydroxychloroquine for COVID19 will have more or fewer arrhythmias than those not receiving 4-aminoquinoline drugs remains to be seen. Furthermore, acute kidney injury is a well-known complication and predictor of mortality of severe malaria \[87\] (for which chloroquine is no longer a first-line treatment because of resistance). Acute kidney injury also occurs in COVID19, and hypokalaemia and hypophosphataemia are common. In view of these uncertainties, monitoring for cardiovascular adverse events (QRS widening, QT prolongation, arrhythmias) and modifiable risk factors (i.e. potassium/calcium/phosphate/magnesium plasma concentrations and severely impaired renal function) is advisable in COVID19 patients receiving high doses of chloroquine or hydroxychloroquine.

Chloroquine and hydroxychloroquine improve glycaemic control in patients with type 2 diabetes, and may occasionally cause hypoglycaemia \[88, 89\]. Several factors contribute: stimulation of insulin secretion, reduced insulin degradation, and increased receptor binding. Pruritus is particularly troublesome in dark-skinned patients and may be dose limiting \[90\]. Itching is described as a widespread prickling sensation mostly affecting the palms, soles, and scalp which starts within 6 to 24 hours, and may last for several days. It can be very distressing. Antihistamine treatment is not usually very effective \[91\]. Hydroxychloroquine may be associated with less itching. Very rarely chloroquine may cause an acute and self-limiting neuropsychiatric reaction \[91, 64\]. In long term use of chloroquine the main concern has been toxicity to the retina. Cumulative doses over 100g (>5 years prophylaxis) are associated with an increased risk of retinopathy \[92, 97\]. Retinal signs include a pale optic disc, arteriolar narrowing, peripheral retinal depigmentation, macular oedema, retinal granularity and oedema, and retinal pigmentary changes consisting of a circle of pigmentation and central pallor; the so called “doughnut” or “bull’s eye” macula. Reversible corneal opacities can be seen in 30-70% of rheumatology patients within a few weeks of high dose treatment. Half are asymptomatic but others may complain of photophobia, visual halos around lights, and blurred vision. Hydroxychloroquine has been considered slightly safer than chloroquine in terms of retinal toxicity, although with sensitive techniques retinal damage is evident earlier than appreciated previously \[96, 97\]. Myopathy is rare at the doses used in antimalarial prophylaxis. Long term high dose use in rheumatological conditions may cause skeletal or cardiac myopathy. The most commonly reported manifestations (85%) are conduction disturbances but some patients develop a hypertrophic restrictive cardiomyopathy leading to congestive heart failure) \[93, 99\]. Less common cutaneous side effects include lightening of skin colour, various rashes (phototoxic dermatitis, exacerbation of psoriasis (severe psoriasis is probably a contraindication), bullous pemphigoid, exfoliative dermatitis, pustular rash), skin depigmentation (with long term use), and hair loss \[64\].
8 Drug interactions

Although chloroquine and hydroxychloroquine are metabolised slowly by several of the cytochrome P450 subfamilies (2C8, 3A4/5, 2D6), this is mainly desethylated – and the metabolites are biologically active [13, 15, 43, 44, 100]. Both drugs have some inhibitory activity on these enzymes but this has not led to clinically significant pharmacokinetic interactions. Displacement may occur, and this probably explains the moderate elevation in primaquine concentrations when coadministered with chloroquine [20]. The main concern has been pharmacodynamic interactions with other hERG channel blocking (QT prolonging) drugs – notably azithromycin which may be coadministered with high dose hydroxychloroquine or chloroquine in COVID19 treatment [56, 101]. Caution should be exercised if such drugs are added, plasma potassium should be over 4mmol/L, calcium and magnesium plasma concentrations should be in the normal range, and electrocardiographs should be monitored. As the 4-aminoquinolines are very slowly eliminated these cautions should continue for days after the drugs have stopped.

9 Chloroquine poisoning

Chloroquine and hydroxychloroquine are dangerous in overdose [47, 100, 102–109]. In self-poisoning, nausea, vomiting, diplopia, hypoacusis, and dysphoria are sometimes followed by tremors, athetoid movements, dysarthria, difficulty swallowing, lethargy and drowsiness and then seizures, coma, hypotension, arrhythmias and ventricular fibrillation. The lethality of chloroquine in overdose is over six times higher than with other drugs [109]. Outcome is dependent on the dose retained, the blood concentrations which result, and the delay in reaching intensive supportive care. Both drugs are absorbed extensively by activated charcoal. In overdose a variety of arrhythmias have been observed including sino-atrial and atrioventricular block, bundle branch block, and different ventricular arrhythmias (including torsade de pointes). The electrocardiogram commonly shows QRS widening and QT prolongation. Hypokalaemia is an important complication, an indicator of prognosis, and a contributor to arrhythmias [100]. It has been suggested that diazepam is a specific antidote [102], but more recent studies do not support a specific role for this drug above good haemodynamic and ventilatory support [104, 105]. During an outbreak of chloroquine self-poisoning in France in the early 1990s, French intensivists rapidly became experienced in the management of chloroquine toxicity. There was loose relationship between the dose self-administered and the resulting blood concentrations. Mortality is proportional to peak blood concentrations. In Clemessy’s large series, only one of 106 overdose patients with a peak blood chloroquine concentration of less than 25 µmol/L died compared with 13 (21%) deaths in 61 patients with peak concentrations above this value [104]. Hypotension, arrhythmias, coma, and ARDS all contributed to fatal outcomes. A fatal outcome was associated with hypotension on admission (Systolic BP <80mm Hg), and an ECG QRS interval >120 milliseconds. Several patients presented with cardiac arrest, and in some others this occurred after thiopental administration (preceding intubation). Clinical evidence of pulmonary oedema was usually absent on admission. In those patients who did develop ARDS it occurred a mean of 17 hours after admission to the ICU [104]. The potassium concentration on admission correlated inversely with QRS widening and QT prolongation. Hypokalaemia (resulting from intracellular accumulation) should be corrected immediately in chloroquine overdose. Good intensive care with careful management of hypokalaemia were important contributors to survival [106, 108]. There is less information on hydroxychloroquine in overdose but the complications and the management are similar. Chloroquine and hydroxychloroquine should be stored in secure containers out of reach of children. Chloroquine should not be prescribed to patients with a history of suicide or those who have suicidal ideas.

In chloroquine poisoning plasma potassium concentrations must be kept above 4mmol/L and plasma magnesium concentrations above 0.8mmol/L.

We pooled individual patient data extracted from [100, 102] (the two largest cohorts available) and estimated the probability of death for the admission chloroquine concentrations (Figure 5). Admission concentrations above 10µmol/L were associated with a greater than 5% chance of dying. We note that this estimate is conservative: admission whole blood chloroquine concentrations will underestimate the peak concentrations.
Figure 5: Relationship between whole blood concentrations of chloroquine measured on admission in self poisoning, and outcome. Top panel: pooled patient data extracted from [100, 102] shown as overlapping histograms for survivors (blue) and non-survivors (red). The overlapping areas are shown by the intermediate colour. Bottom panel: estimated mean probability of death as a function of the admission whole blood concentrations of chloroquine. Grey area represents 95% confidence interval. The dashed line shows the value of 5% probability.
Figure 6: Pharmacokinetic treatment profiles for a typical adult population. Simulated whole blood concentration-time profiles of chloroquine (n=1,000; 75 kg), based on [15, 10]. Solid black line shows the population mean concentration-time profile and the shaded area shows the 90% prediction interval. Red dashed line indicates a putative IC50 value for SARS-CoV-2, scaled to total blood concentrations (chloroquine: 5.65 μM; hydroxychloroquine: 2.88 μM, using reported in vitro IC50 values [30, 58] and a blood:plasma ratio of 5:1 for chloroquine [14] and 4:1 for hydroxychloroquine [39]).

10 Dosing simulations of chloroquine and hydroxychloroquine

10.1 Treatment simulations

We simulated a treatment scenario consisting of a loading dose of 4 tablets of hydroxychloroquine sulphate (each tablet 200mg equivalent to 155mg base) or chloroquine phosphate (each tablet 250mg equivalent to 155mg base) at time 0 and 6 hours, followed by a maintenance dose of 2 tablets twice daily (starting 12 hours after first dose) for a total treatment duration of 7 or 10 days (Figure 3). These are the doses currently being evaluated in the large SOLIDARITY and RECOVERY randomised trials (registered in the ISRCTN registry, numbers 50189673 and 83971151). The reported in vitro inhibition EC50 values for SARS-CoV-2 (1.13μM for chloroquine [58] and 0.72μM for hydroxychloroquine [30]), scaled to whole blood concentrations, are shown as an indicator. The degree of protein binding in the laboratory system medium is unknown. Thus, the reported in vitro values were assumed to correspond to total plasma values and scaled to whole blood using a reported blood:plasma ratio of 5:1 for chloroquine [14] and 4:1 for hydroxychloroquine [39], resulting in a putative in vivo blood IC50 value of 5.65μM and 2.88μM for chloroquine and hydroxychloroquine, respectively.

To evaluate exposure and peak concentration after treatment of patients with different body weights (40-90 kg), we simulated three different dosing strategies (Figure 7):

1. a flat dosing scenario,
2. a weight-based loading dose followed by a flat maintenance dose, and
3. a weight-based loading dose and weight-based maintenance dose.

Flat dosing (1) consisted of the 7-day treatment scenario described above (loading dose of 4 tablets, followed by a maintenance dose of 2 tablets). The weight-based loading dose (2) used 3 to 5 tablets to achieve an individual loading dose as close as possible to 10 mg base/kg for patients at different body weights, followed by a flat maintenance dose of 2 tablets to all patients.
final scenario was to develop both a weight-based loading dose (same as above) and a weight-based maintenance dose. The maintenance dose was between 1 and 3 tablets to achieve a maintenance dose as close as possible to 5 mg/kg (Figures 8 and 9). Predictably, weight-based loading and maintenance dosing is preferable in underweight or overweight patients.

10.2 Prophylactic treatment simulations
In the simulations of prophylactic dosing over three months, whole blood exposure and peak concentrations at different body weights (40-90 kg), were simulated using a weight-based loading dose of 3-5 tablets (Figure 10) followed by a flat maintenance dose of 1 tablet daily (Figure 11). These show expected exposures as in the treatment of rheumatological conditions which are well below those associated with cardiovascular safety concerns, but it remains to be seen if these levels are effective in the prevention of COVID19.

10.3 Renal impairment
Renal clearance of chloroquine and hydroxychloroquine has been reported to be between 20% and 55% [18, 31]. A “worst-case” scenario was simulated in which renal clearance was reduced by 90% with no compensatory increase in hepatic metabolism. Thus, total body clearance was reduced with 45%. Both acute treatment and prophylactic therapy was simulated, assuming no adjustment in loading dose, and an alternative dosing of half the maintenance dose in patients with renal impairment (Figure 12). Whole blood chloroquine exposure was similar in patients with adequate renal function and renal impairment in short-course treatments of 7 or 10 days. Dose adjustment will not be needed in these patients. Exposures are significantly higher in patients with renal impairment receiving prophylactic treatment. It is uncertain whether this requires dose modification.

11 Discussion
11.1 The case for evaluating large doses in COVID19 infections?
The inhibitory activity of chloroquine and hydroxychloroquine against the SAR-CoV-2 virus is seen only at high concentrations in vitro so if there is any benefit from these medicines it is likely to require high concentrations of free drug in blood to drive high concentrations in the infected respiratory epithelium. The concentration effect relationships derived from studies in Vero cells ex vivo are likely to be only a loose guide to activity in vivo, but if they are relevant they suggest only partial antiviral effects at best (as shown in Figures 6 & 12). We assume that the cytosolic
Figure 8: Chloroquine pharmacokinetic exposure after treatment. Simulated exposure parameters of whole blood chloroquine, stratified by body weight (n=1,000 per body weight), based on [15]. Solid black line shows the population mean exposure and the shaded area shows the 90% prediction interval. Black dashed line indicates exposure associated with a standard dosing of 10 mg base/kg loading dose followed by 5 mg base/kg maintenance dose (i.e. exposure in a patient weighing 62 kg). AUC: area under the concentration-time curve from time zero to one month after the last dose. \( C_{\text{MAX}} \) is the maximum concentration.
Figure 9: Hydroxychloroquine pharmacokinetic exposure after treatment. Simulated exposure parameters of whole blood hydroxychloroquine, stratified by body weight (n=1,000 per body weight), based on [110]. Solid black line shows the population mean exposure and the shaded area shows the 90% prediction interval. Black dashed line indicates exposure associated with a standard dosing of 10 mg/kg loading dose followed by 5 mg base/kg maintenance dose (i.e. exposure in a patient weighing 62 kg). AUC: area under the concentration-time curve from time zero to one month after the last dose. $C_{MAX}$ is the maximum concentration.
concentrations of the drugs in the respiratory epithelium will be in dynamic equilibrium with the free fraction in plasma. We know from the treatment of life-threatening infections that the earlier in the evolving disease process that pathogen multiplication is inhibited the better the outcome. This argues for achieving high blood concentrations of chloroquine or hydroxychloroquine as soon as possible, but this has to be balanced against the potential for serious toxicity. This is exactly the same argument used in the treatment of malaria [5] which in the “chloroquine era” had a mortality of 0.1% for uncomplicated infections but 15-20% in severe disease. The doses being evaluated in the two largest COVID19 randomised controlled treatment trials are certainly high (loading doses of 10mg base/kg at 0 and 6 hours followed by 5mg base/kg twelve hourly), but the disease can be serious. The concentrations that will occur in the first few hours of treatment are similar to those which will occur after about one week’s twice daily treatment (see simulated profiles in Figure 6). After loading, the doses are then reduced to maintain high blood levels. A misunderstanding of the pharmacokinetic properties of chloroquine may already have led to premature discontinuation of one study in which “loading doses” of 10mg base/kg were continued twice daily for ten days and lethal cardiotoxicity ensued [111]. Although there is little experience to date in the treatment of COVID19 the extensive previous experience in acute malaria suggests that serious toxicity (hypotension, arrhythmias, convulsions) should be unlikely with the high dose regimens being evaluated in the larger randomised trials. The relationship between admission whole blood chloroquine concentrations and outcome in intentional overdose (Figure 5) provides additional reassurance that life-threatening cardiovascular toxicity is unlikely with these regimens. In the treatment of malaria, sicker bed-bound patients generally tolerate these drugs relatively well whereas ambulant febrile patients may feel nauseated and temporarily dysphoric. It is hoped that COVID19 patients would respond in a similar way to malaria patients but this will need to be determined.

For prevention or prophylaxis, where the viral burden is much lower, the doses being recommended are also lower. They are similar to those being used in rheumatological conditions [38, 65, 112, 117]. These are generally tolerated well.
Figure 11: Prophylactic pharmacokinetic profiles of chloroquine and hydroxychloroquine. Simu-
lated whole blood concentration time profiles of chloroquine and hydroxychloroquine (n=1,000 per
body weight group), based on [15, 110]. Solid black line shows the population mean concentration-
time profile and the shaded area shows the 90% prediction interval. Red dashed line indicates a
putative IC$_{50}$ value for SARS-CoV-2, scaled to total blood concentrations (chloroquine: 5.65 µM;
hydroxychloroquine: 2.88 µM, using reported in vitro IC$_{50}$ values [30, 58] and a blood:plasma ratio
of 5:1 for chloroquine [14] and 4:1 for hydroxychloroquine [39]).
Figure 12: Simulated blood concentration time profiles of chloroquine and hydroxychloroquine (n=1,000), based on [15, 110]. Solid black line shows the population mean concentration-time profile in a patient with normal renal clearance after standard maintenance dose. Solid red line shows the population mean concentration-time profile in a patient with renal impairment (10% of renal function) after standard maintenance dose. Solid blue line shows the population mean concentration-time profile in a patient with renal impairment (10% of renal function) after half of standard maintenance dose. Black dashed line indicates the maximum concentration in a typical patient. Red dashed line indicates a putative IC$_{50}$ value for SARS-CoV-2, scaled to total blood concentrations (chloroquine: 5.65 µM; hydroxychloroquine: 2.88 µM, using reported in vitro IC$_{50}$ values [30, 58] and a blood:plasma ratio of 5:1 for chloroquine [14] and 4:1 for hydroxychloroquine [39]).
11.2 Should chloroquine or hydroxychloroquine be given empirically for the prevention or treatment of COVID19?

Many argue that, given the gravity and impact of COVID19 on individuals, families, organisations and society, having “something is better than nothing”. Giving chloroquine or hydroxychloroquine as a prophylaxis for COVID19 allays anxiety and gives hope to millions of people at risk of contracting the disease. Giving a treatment to a seriously ill patient gives the impression something potentially beneficial is being done. And if these drugs do work, then lives would have been saved, and individuals at risk would have been protected. We outline the arguments against this position from an evidence-based medicine perspective, which has largely replaced opinion-based medical decision making. We do not know now if giving chloroquine or hydroxychloroquine for the prevention or treatment of COVID19 is better, or worse, than nothing.

Lack of evidence There is no evidence that chloroquine and hydroxychloroquine or indeed any pharmaceutical intervention is effective in the prevention or treatment of COVID19. The global standard for determining efficacy is through well conducted randomised clinical trials (RCTs). There have been some small treatment trials with chloroquine and hydroxychloroquine reported, and no RCTs yet in prevention, although several large trials are underway. We are clearly in equipoise, awaiting the results of large and definitive studies (and trying to sustain randomised trials under bombardment from the media which is fuelled by a steady stream of rushed and often underpowered reports) [117]. Recommending the use of chloroquine or hydroxychloroquine before their safety and efficacy have been determined compromises these planned critical trials and it violates the generally accepted principle of recommending interventions only after there is sufficient evidence of their safety and efficacy.

Risk versus benefits Chloroquine and hydroxychloroquine are not harmless. Even though we do not know if they are harmful or beneficial in prevention or treatment of COVID19, some countries now recommend them both in treatment and in prophylaxis for high risk groups [118,119]. A national recommendation based on inadequate evidence is irresponsible and it gives the public the wrong message. The public who are desperate for a “cure” will often not read the fine print - or they will believe the message that “something is better than nothing”. This could also lead to widespread self-medication. Fatal self-poisonings have already been reported from unsupervised self-medication of chloroquine and hydroxychloroquine in several countries [119].

Diversion Recommending valuable drugs for unproven indications wastes valuable resources, damages health, and compromises finding effective medicines. People are likely to assume that recommended drugs do work and, in the context of preventive use, will believe they are protected and therefore may not take other necessary precautions or adhere to other public health measures. Taking drugs is easier than complying with public health measures such as social distancing and wearing protective equipment. In addition, the high demand for these currently unproven drugs puts patients who legitimately need them for treatment for other conditions such as SLE and rheumatoid arthritis at risk. Shortages have already occurred and prices have risen markedly leaving these vulnerable groups to suffer unnecessarily [120]. It could also encourage unscrupulous manufacturers to make falsified chloroquine and hydroxychloroquine [121]. This is already happening.

How can we find the answer? Well conducted RCTs are needed [117]. Premature national recommendations for chloroquine and hydroxychloroquine use may compromise planned clinical trials to determine their benefit [122] and may make recruitment into these trials more difficult. In randomized trials sick patients and their relatives will want to know why they are being denied treatments advocated or recommended elsewhere. Also, potential participants in pre or post exposure trials may opt for self-medication rather than join the trials. This jeopardises the equipoise between the drug and no intervention, at least in the eyes of the public. And what are the criteria for countries (such as India and Bangladesh) to stop recommending chloroquine chemoprophylaxis for health care workers? Are they waiting for results from elsewhere, or will they continue indefinitely?
Undermining the drug regulatory system  Recommending chloroquine and hydroxychloroquine for widespread prophylaxis use is not the same as getting approval for unproven drugs for compassionate use. This is a global problem and it needs the best science to counter it. Unjustified recommendations based on opinion or politics rather than evidence undermines public trust in the regulation of the pharmaceutical industry and goes against the time-tested drug approval and regulatory mechanisms established over a generation to protect public safety.
12 Methods

12.1 Pharmacokinetic modelling

The population pharmacokinetic models published to date, describing chloroquine and hydroxychloroquine, are limited and do not capture fully the terminal elimination half-life or the total apparent volume of distribution of these drugs. Few studies have collected blood samples for longer than 30 days after the last dose, and therefore there are insufficient data to describe adequately the complex multi-compartment kinetic properties of these drugs. The resulting 1- or 2-compartment models underestimate the true terminal elimination half-life. This limitation was highlighted by Frisk-Holmberg and colleagues [15] who reported an increasing terminal half-life when data were quantified for up to 250 days after dose, resulting in a doubling of the estimated terminal elimination half-life (27 vs 53 days) as more data were included in the analysis. This results in overestimation of the time to reach steady state. However, these simplified (mis-specified) models should predict the concentration-time profiles relatively well during short treatment courses, provided the drugs are not absorbed rapidly enough to outpace distribution from the “central compartment” [19]. The terminal elimination phase has little impact on the acute treatment blood concentration profiles. However, these simplified models are inaccurate when simulating long-term prophylactic regimens. We therefore used data collected for a long duration after a single dose in the pharmacokinetic simulations, in order to characterise both the complex distribution of these drugs and their slow elimination. Two small studies in healthy volunteers were selected for chloroquine [15] and hydroxychloroquine [110], both reporting a 3-compartment disposition model resulting in a long terminal half-life of 1-2 months. Estimated mean pharmacokinetic parameters where used to simulate published short course treatments [36, 123] and repeated dosing to steady-state [30, 124], to ensure that model-derived simulated concentrations captured unbiased drug measurements reported in the literature. In the case of a deviation, the relative bioavailability was used to scale the model. Parameter estimates are presented in the table below Additional between-patient variability was added exponentially to the model to generate the reported variability in peak concentrations (i.e. 35% between-patient variability in all parameters). The final pharmacokinetic model parameters with between-patient variability are shown in Table 3. All models were coded and simulated using the pharmacokinetic software NONMEM v.7.3 (Icon Development Solution, Ellicott City, MD). Whole blood simulations were compared to a reported \textit{in vitro} IC$_{50}$ value for SARS-CoV-2 of 1.13µM for chloroquine [58] and 0.72µM hydroxychloroquine [30]. These indicative values were assumed to correspond to total plasma values and scaled to whole blood using a reported blood-plasma ratio of 5:1 for chloroquine [14] and 4:1 for hydroxychloroquine [39], resulting in a putative \textit{in vivo} blood IC$_{50}$ value of 5.65µM and 2.88µM for chloroquine and hydroxychloroquine, respectively.

12.2 Pharmacokinetic-pharmacodynamic modelling of chloroquine poisoning

We conducted a literature review of all published case reports and hospital cohorts on chloroquine poisoning. We extracted data from 12 case reports (total of 13 patients) for which chloroquine concentrations were given. We extracted graphical data from 3 large cohorts [100, 102, 104] whereby chloroquine concentrations (whole blood in the three studies) and outcome could be determined. A total of 176 unique observations could be reliably extracted from Clemessy et al. [100]; 133 unique observations from Clemessy et al. [104]; and 102 unique observations from Riou et al. [102]. The extracted data are provided in the supplementary materials. The two Clemessy studies reported overlapping patient cohorts so we used only the larger cohort [100]. Data from the case reports exhibited significant bias towards patients with high concentrations who survived. We included only case reports in which blood or plasma concentrations were obtained ante-mortem as the post-mortem redistribution of chloroquine to the blood is unknown. Our final pharmacodynamic model pooled data from Riou et al. (1988) [102] and Clemessy et al. (1995) [100], giving a total of 278 unique observations. Data were extracted using the web version of WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/).

We modelled the probability of death given the admission whole blood chloroquine concentration (units were µmol/L) using a logistic regression model with the concentration on the log base 10 scale. In both publications, whole blood chloroquine concentrations were determined using ultraviolet spectrophotometry at a wavelength of 343nm. This method does not differentiate between
Table 3: Pharmacokinetic parameters for chloroquine and hydroxychloroquine, used for simulating different dosing scenarios. CL/F is the apparent elimination clearance, $V_c/F$ is the apparent volume of distribution of the central compartment, $Q/F$ is the apparent intercompartmental clearance between the central and first peripheral compartment, $V_{p1}/F$ is the apparent volume of distribution of the first peripheral compartment, $Q_2/F$ is the apparent intercompartmental clearance between the central and second peripheral compartment, $V_{p2}/F$ is the apparent volume of distribution of the second peripheral compartment, $k_a$ is the absorption rate constant, $T_{lag}$ is the lag time in the absorption phase and $F$ is the relative bioavailability. Between-patient variability, 35%, were added exponentially in all parameters.

Chloroquine and the desethyl metabolite. However, this should not impact the generalizability of the results, as in poisoning, the short period between ingestion and measurement means that little metabolism has occurred, and the biological effects of the desethylated metabolites are considered similar to the parent compounds. The predicted relationship between whole blood chloroquine concentrations and death estimated from these data will be conservative. Chloroquine concentrations were measured on admission, but these will often be lower than the peak concentration. However, the estimated relationship allows for an objective evaluation of the risk of toxicity in high concentrations.

References


