Pharmacogenomics and Personalized Medicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

REVIEW

Repurposing Drugs for COVID-19: Pharmacokinetics and Pharmacogenomics of Chloroquine and Hydroxychloroquine

This article was published in the following Dove Press journal:
Pharmacogenomics and Personalized Medicine

Mariana Babayeva[1]
Zvi Loewy[1,2]

[1]Touro College of Pharmacy, New York, NY, USA; [2]New York Medical College, Valhalla, NY, USA

Background: A new coronavirus SARS-CoV-2 has been identified as the etiological agent of the severe acute respiratory syndrome, COVID-19, the source and cause of the 2019–20 coronavirus pandemic. Hydroxychloroquine and chloroquine have gathered extraordinary attention as therapeutic candidates against SARS-CoV-2 infections. While there is growing scientific data on the therapeutic effect, there is also concern for toxicity of the medications. The therapy of COVID-19 by hydroxychloroquine and chloroquine is off-label. Studies to analyze the personalized effect and safety are lacking.

Methods: A review of the literature was performed using Medline/PubMed/Embase database. A variety of keywords were employed in keyword/title/abstract searches. The electronic search was followed by extensive hand searching using reference lists from the identified articles.

Results: A total of 126 results were obtained after screening all sources. Mechanisms underlying variability in drug concentrations and therapeutic response with chloroquine and hydroxychloroquine in mediating beneficial and adverse effects of chloroquine and hydroxychloroquine were reviewed and analyzed. Pharmacogenomic studies from various disease states were evaluated to elucidate the role of genetic variation in drug response and toxicity.

Conclusion: Knowledge of the pharmacokinetics and pharmacogenomics of chloroquine and hydroxychloroquine is necessary for effective and safe dosing and to avoid treatment failure and severe complications.

Keywords: COVID-19, pharmacokinetics, pharmacogenomics, chloroquine, hydroxychloroquine

Introduction

SARS-CoV-2 is a new coronavirus type that has not been previously identified in humans. Little is known about the highly infectious virus or how to combat it. The current strategy considers two broad categories of therapies: antivirals, which may target the coronavirus directly, and host modifiers and immune-based medications, which may influence the immune response to the virus. Currently, all the therapeutic agents are repurposed medications.

For approximately 6000 identified medical conditions, only 500 have approved therapies; a critical need currently exists for the availability of drug therapies.[1–3] Drug repurposing helps to minimize the deficiency and delivers a candidate at a shorter development time and a lesser cost.[4] A key advantage of repurposed drugs

Correspondence: Mariana Babayeva
Touro College of Pharmacy, 230 West 125th Street, Room 433, New York, NY 10027, USA
Tel + 1 646 981 4740
Fax +1 212 678 1780
Email mariana.babayeva@touro.edu

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/PGPM.S275964

is that safety has been established and only efficacy of the new indication needs to be assessed.[5] Many of the well-known repurposed drugs, including sildenafil, minoxidil and aspirin emerged by chance from "unorganized" drug discovery processes.[6] Several diverse disease states have common transcriptional inflammatory and metabolic pathways, suggesting that drugs designed for treatment of one disease can potentially be used to treat other diseases.[7] Drug repurposing is being applied to finding a therapeutic approach for the COVID-19 pandemic. Thirty-one potential broad-spectrum antiviral agents (BSAAs) were recently identified as having potential for treating SARS-CoV-2/COVID-19.[8] Several existing BSAAs have been initiated into clinical trials (Table 1).

Two of the drugs listed in Table 1, hydroxychloroquine and chloroquine, have been proposed as treatments for COVID-19.

Chloroquine (Aralen®) and hydroxychloroquine (Plaquenil®) are 4-aminoquinoline medications used to treat several disease states. Chloroquine (CQ) was first developed for the treatment of malaria.[9] Hydroxychloroquine (HCQ) is β-hydroxylated analogue of CQ.[10] Both medications have been successfully used to treat extraintestinal amebiasis, several infectious (HIV, Q fever, Zika virus, fungal infections) and rheumatological (systemic lupus erythematosus, antiphospholipid syndrome, rheumatoid arthritis, Sjögren's syndrome) diseases.[11–13]

In the therapy of malaria, the agents inhibit the action of heme polymerase, which causes the buildup of toxic heme in *Plasmodium* species.[14,15] The antiviral activity is not fully understood. The drugs accumulate in human organelles, raise the endosomal pH and prevent viral activity.[16–18] The elevated pH inhibits nucleic acid replication, glycosylation of viral proteins, viral fusion and entry into the cell, viral assembly and release.[19]

Table 1 Broad-Spectrum Antiviral Agent Candidates in Clinical Trials

Phase II Favipiravir
Phase III Remdesivir Hydroxychloroquine Chloroquine
Phase IV Umifenovir Lopinavir/Ritonavir

Adverse Reactions of Chloroquine and Hydroxychloroquine

Postmarketing cases of life-threatening and even fatal events have been reported for chloroquine and hydroxychloroquine. An overdose of CQ can cause acute poisoning and death.[20] HCQ was demonstrated to be 40% less toxic than chloroquine, although prolonged and overdose administration can still cause poisoning.[21,22] Patients may present with atrioventricular block, pulmonary hypertension, sick sinus syndrome or with cardiac complications. The most life-threatening adverse reaction is QTc prolongation with subsequent risk of ventricular arrhythmias.[23] Concomitant QTc-prolonging medications may increase the severity of the complication even more.[24] The mechanism of QTc prolongation by chloroquine and hydroxychloroquine is unknown, largely depending on the cardio-vascular health of the patients.[25–27] Another complication of the two medications is retinopathy (*chloroquine retinopathy*), which can result in irreversible impairment of the retina.[28,29] High concentrations of the drugs in the retina, due to binding to retinal melanin, result in the damage of the tissue.[30] Hydroxychloroquine may also produce a severe cutaneous adverse effect such as a generalized pustular figurate erythema (GPFE).[126] High concentrations of the medications in the skin and very slow cutaneous elimination (longer than 6 months) may result in this severe cutaneous reaction.[59,87] The most common adverse effects of the drugs are nausea, vomiting, diarrhea.[31] Other complications include hypoglycemia in diabetics, hemolytic anemia in G6PD deficiency patients, tinnitus and headache.[32,33]

Chloroquine and Hydroxychloroquine for COVID-19 Therapy

Chloroquine and hydroxychloroquine have shown the ability to inhibit replication of multiple coronaviruses in vitro,[34–36] including SARS-CoV-2 in concentration-dependent manner.[17,19,22,37,38] The anti-SARS-CoV-2 activity of HCQ seems to be less potent compared to CQ. The EC_{50} for CQ (2.71 μM) was significantly lower than that of HCQ (4.51μM).[22] In contrast, hydroxychloroquine was found more potent than chloroquine against SARS-CoV2 when given post-infection and prophylactically.[19]

Clinical evidence of the effectiveness of HCQ or CQ for the treatment of COVID-19 is limited. Some small clinical trials have shown therapeutic benefits of the drugs, while others have shown the opposite. In recent clinical trials, over 100 people with COVID-19 have been treated with

chloroquine. These patients had less severe disease and a shorter illness duration compared to those who did not receive chloroquine.[39] Another open-label non-randomized clinical trial with 36 COVID-19 patients demonstrated that hydroxychloroquine treatment resulted in viral load reduction/disappearance in the patients. The effect was reinforced by azithromycin.[40] Contrasting results were reported in a small study with 11 hospitalized patients; no difference in clinical outcomes was observed between patients treated with HCQ and azithromycin and patients on standard care.[41] In a randomized trial with 62 hospitalized patients, patients on HCQ had a more substantial proportion of clinical improvement of pneumonia (80% vs 55%) than patients with standard care.[42] In another clinical trial with 368 COVID-patients, an increased overall mortality was observed in the patients treated with hydroxychloroquine.[43] More clinical trials are going on.

The FDA has issued an emergency use authorization for CQ and HCQ to treat COVID-2019 infection, allowing the unapproved use of these medications in light of a public health emergency.[32,33] On April 24, the FDA issued warning against HCQ or CQ unless the therapy is closely supervised by a healthcare professional.[44] The caution was initiated after the agency received reports of serious adverse effects in COVID-19 patients. These findings do not apply to the use or evaluation of hydroxychloroquine in pre- or post-exposure prophylaxis in patients exposed to COVID-19.

These results bring forward the need for large controlled clinical trials to provide guidance on safe and effective dosing of CQ/HCQ for COVID-19 therapy. Furthermore, response to drugs is subject to inter-individual variability. Patients treated with the same dose of the same drug, may exhibit lack of efficacy, or adverse reactions. The variability, at least in part, is attributed to genetic polymorphisms. Knowledge of pharmacogenomic (PGx) of the drugs is necessary to estimate effective and safe dosing and to avoid/minimize adverse reactions. No PGx studies have been conducted to investigate the inter-patient variability of CQ and HCQ in COVID-19 patients.

The purpose of the study was to highlight the importance of large, randomized, controlled clinical trials and pharmacogenomic studies to assess the optimal dosing of CQ and HCQ in diverse populations as a treatment for COVID-19.

Methods

A review of the literature was performed using Medline/PubMed/Embase database resources for English language papers from 1947 up to July 2020 to identify appropriate articles that addressed the objectives of this review. A variety of keywords were employed in keyword/title/abstract searches that included: chloroquine, CQ, hydroxychloroquine, HCQ, pharmacokinetics, pharmacogenomics, COVID-19, SARS-CoV-2. We obtained 126 appropriate results after screening all sources; relevant and non-relevant. The publications were reviewed independently by two investigators. The investigators extracted the data and inspected each reference identified by the search. In cases where the same studies were reported in more than one publication, the study's results were accounted for only once. Limits to the search strategy were English language articles and human studies. The electronic search was followed by extensive hand searching using reference lists from the identified articles. The search method was used to strengthen existing concepts and to identify study designs for upcoming research studies.

Results

Pharmacokinetics of Chloroquine and Hydroxychloroquine

This paper presents the current knowledge on chloroquine (CQ) and hydroxychloroquine (HCQ) pharmacokinetics (PK) with a focus on stereoselectivity of their disposition. Both drugs are racemic mixtures, consisting of equal amounts of R(-) and S(+)-enantiomers.[45] The pharmacokinetics of these two 4-aminoquinolines are similar and regulate dosing of the drugs.

Dosing Considerations

CQ is available as chloroquine phosphate, Aralen®. Each 500-mg tablet of chloroquine phosphate contains 300 mg of chloroquine. HCQ is available as hydroxychloroquine sulfate, Plaquenil®. Each 200-mg tablet of hydroxychloroquine sulfate contains 155 mg of hydroxychloroquine. Doses of both agents are based on ideal body weight (IBW). Chloroquine doses are 3.5–4.0 mg/kg/day and produce plasma levels of 6 to 9×10^{-7} M/L. Doses of hydroxychloroquine are 6.0–6.5 mg/kg/day and produce plasma concentrations of 1.4 to 1.5×10^{-6} M/L.[46] An initial adult dose of chloroquine phosphate for malaria therapy is 1 g followed by 500 mg given at 6–8 hours, 24, and 48 hours. Lupus erythematosus and rheumatoid arthritis chloroquine phosphate dosage is 250 mg daily with dose reduction after remission.[9]

Initial adult dose of hydroxychloroquine sulfate is 800 mg followed by 400 mg given at 6–8 hours, 24, and

48 hours. Lupus erythematosus and rheumatoid arthritis initial treatment is 400–600 mg of hydroxychloroquine sulfate daily for several weeks or months.[10]

Pediatric dosage of chloroquine and hydroxychloroquine is based on body weight. An initial pediatric dose of chloroquine phosphate for treatment of malaria is 16.7 mg/kg followed by 8.3 mg/kg given at 6, 24, and 48 hours after initial dose. Maximum total dose is 2.5 g.[9] Initial pediatric dose of hydroxychloroquine sulfate for treatment of malaria is 12.9 mg/kg followed by 6.4 mg/kg given at 6, 24, and 48 hours after the first dose. Maximum total dose is 2 g.[10]

CQ and HCQ have been used for prophylaxis and treatment of malaria in pregnant women without evidence of adverse effects on the fetus. Dosing for treatment and prophylaxis of uncomplicated malaria is the same in pregnant and nonpregnant adults. Due to pregnancy-induced physiologic changes, some pharmacokinetic properties of the drugs may be altered, suggesting dose adjustments may be needed. But data are not sufficient to determine an appropriate dosing during pregnancy.[9,10]

Small amounts of chloroquine and hydroxychloroquine excrete into breast milk. The amounts of the drugs are not sufficient to harm the infant nor to protect the child from malaria. Weekly CQ/HCQ of 500/400 mg may be given until breastfeeding is completed.[9,10]

No information is available on the effect of chloroquine and hydroxychloroquine in geriatric patients. But because CQ and HCQ are mostly excreted in the urine, elderly patients with age-related kidney problems may require caution and a dose adjustment for the patients. The dose adjustment should be based on the kidney function.

The optimal dosing of HCQ and CQ for treatment of COVID-19 is unknown. Most of the published clinical studies had HCQ dosage of 400 mg/5 days or 800 mg on the first day and 400 mg for the next 4 days. The latest regimen was supported by pharmacokinetic modelling, where an oral HCQ sulfate loading dose of 400 mg twice daily, followed by a maintenance dose of 200 mg twice daily for 4 days was able to achieve treatment efficacy and a good safety profile.[19] This regimen reached three times the potency of CQ phosphate given 500 mg twice daily for 5 days.[19] However, more reliable information is required before it can be widely used to treat COVID-19.

Absorption

Oral absorption of chloroquine and hydroxychloroquine in humans is efficient. Both drugs have oral bioavailability of 0.7–0.8.[47,48] Although 2-3-fold difference in the absorbed fraction of oral doses was reported.[49,50] Maximum blood concentrations (Cmax) for the oral doses showed significant differences between subjects (range 135–422 ng/mL), but not within subjects.[51,52] Oral bioavailability of chloroquine is 52–114%. CQ oral tablets have slightly greater bioavailability than oral solutions, 67–114% vs 52–102%, respectively.[16] Hydroxychloroquine has oral bioavailability of 67–74%.[47,53] Antacids may decrease the bioavailability of both drugs.[54] Oral chloroquine reaches Cmax faster than hydroxychloroquine. Time to reach the maximum level (Tmax) for CQ was estimated at 30 minutes,[16,55–57] while Tmax for HCQ was estimated 3.74 hours.[47,58] Absorption of the R and S enantiomers was not significantly different.[47]

Distribution

CQ and HCQ have multicompartment disposition in humans with wide distribution to the body tissues.[48] Highest concentrations were found in the melanin-containing cells, the retina and the skin. High levels were observed in the liver, spleen, kidney, and lung.[59] In the blood, concentrations in erythrocytes were up to 5 times higher than in plasma.[60] Reported volumes of distribution were 44,000 L and 65,000 L for HCQ and CQ, respectively.[16,57,61–63]

Plasma protein binding of the drugs ranges between 50% and 60%.[47,64] CQ and HCQ are mostly bound to two plasma proteins, albumins and α-1-acid glycoproteins. Binding of both compounds to plasma proteins is stereoselective. Chloroquine is approximately 60% bound to plasma proteins.[16,65] Extend of S(+)-chloroquine plasma protein binding is greater than binding of R(-)-chloroquine (67% vs 35%).[66] Binding of hydroxychloroquine to plasma proteins is around 50%, which is less than chloroquine binding. The S-hydroxychloroquine is 64% bound to plasma proteins, while the R-hydroxychloroquine is only 37% protein bound.[47] Following separate administration of the individual enantiomers of both drugs, R(-)-isomers reach higher and more sustained plasma and ocular concentrations than S(+)-forms.[16,67,68]

Metabolism

Chloroquine and hydroxychloroquine have long half-lives and low blood clearance. CQ is rapidly N-desethylated into two major metabolites: desethylchloroquine (40%) and bisdesethylchloroquine (10%).[69,70] Desethylchloroquine is the pharmacologically active metabolite and further metabolizes to bidesethylchloroquine. CQ is metabolized primarily by CYP2C8 and CYP3A4 mediating 80% of the total

metabolism of the drug. Other enzymes, CYP3A5 and CYP2D6 break down chloroquine to a lesser extent.[69,71–73] Metabolism of CQ is stereoselective. After administration of the individual enantiomers, the concentration of (R)-chloroquine was 1.3-fold higher compared to concentrations of (S)-chloroquine in plasma and 1.8-fold higher in the blood in patients with rheumatoid arthritis.[74] Blood concentrations of the active metabolite S(+)-desethylchloroquine exceeded those of the R(-)-forms.[67,75,76]

HCQ has similar to chloroquine biotransformation but breaks down into more metabolites. HCQ is N-dealkylated by CYP3A4 to the two active metabolites desethylhydroxychloroquine, desethylchloroquine and an inactive metabolite bidesethylchloroquine. Other cytochrome P450 enzymes (CYP2C8, 2D6, and 3A5) are involved in the metabolism to a lesser extent.[64,77] Biotransformation of HCQ is also stereoselective. Several studies have reported faster hepatic metabolism of S(+)-enantiomers compared to metabolism of R(-)-enantiomers.[67,76,78–80] The blood and plasma concentrations of R-hydroxychloroquine exceeded those of the S-hydroxychloroquine with the mean R/S ratio of 2.2 in the blood and 1.6 in the plasma.[81] The mean blood concentration ratio R/S for desethylhydroxychloroquine was 0.45 and for desethylchloroquine was 0.56, indicating stereoselective metabolism of the compound.[81]

Similar doses of the two drugs produced 11-fold variations in the blood concentrations in patients with rheumatoid arthritis[47,63,82,83] and in healthy volunteers,[52,64] suggesting different extend of metabolism among individuals. Moreover, chloroquine and hydroxychloroquine are involved in several metabolic drug–drug interactions (DDIs). A CYP3A4 inhibitor, cimetidine increased serum concentrations of CQ by 48%.[84] Another CYP3A4 inhibitor, ketoconazole reduced the formation of active metabolite desethylchloroquine.[71,85]

Excretion

Urinary excretion is the main route of elimination for chloroquine and hydroxychloroquine.

The 50% of a chloroquine dose is recovered in the urine as unchanged drug, with 10% of the dose recovered in the urine as its active metabolite desethylchloroquine.[16] The 19% of a CQ dose is recovered in feces.[86] Small amounts (5%) of the drug eliminate through the skin and up to 45% stored in lean tissues.[46] Elimination from the skin is very slow. CQ remains in the skin longer than 6 months, a time when the drug is no longer detectable in the plasma.[87] Chloroquine and active metabolite desethylchloroquine have elimination half-lives of 20 to 60 days and may be detected in urine months after a single dose.[16] Chloroquine has a total clearance of 0.35–1L/h/kg.[16] Renal clearance accounts for half of the total systemic clearance and increases by acidification of the urine.[87]

The renal excretion accounts for 40–50% of HCQ elimination, where only 16–21% is excreted as unchanged drug.[47] The 24–25% of absorbed dose is excreted in the feces, which is greater than CQ feces excretion.[46] The elimination through the skin and long-term storage in lean tissues is identical to those of chloroquine, 5% and 45%, respectively.[21,46,88] The total clearance of hydroxychloroquine is 96 mL/min.[47] IV hydroxychloroquine has a half-life of 40 days (22.4 days in blood, and 123.5 days in plasma).[89] The elimination half-life of both drugs is significantly longer in patients with chronic renal disease.[16,57] In anuretic patients, the plasma levels were 70% and 25–30% higher for CQ and HCQ, respectively, compared to concentrations in subjects with normal kidney function.[53] Enantioselective renal elimination of the medications has been demonstrated in patients.[66,74] (S)-hydroxychloroquine had a mean renal clearance approximately twice that of (R)-hydroxychloroquine. In addition, the mean renal clearance of active (S)-metabolites was also higher than that of (R)-metabolites.[81]

The main mechanism of renal elimination of the medications is tubular secretion as renal excretion 7-fold exceeds the glomerular filtration rate.[57,90] The tubular secretion is an active process mediated by membrane proteins. It was reported that chloroquine is a substrate and potent competitive inhibitor of multidrug and toxin extrusion protein 1 (MATE1).[91,92] As substrates and/or inhibitors of active transport and metabolism, CQ and HCQ may be involved in several drug–drug interactions.[93–98]

Pharmacogenomics of Chloroquine and Hydroxychloroquine

Response to drugs is subject to inter-individual variability. 40–70% of individuals that receive a drug, exhibit lack of efficacy, or adverse drug reactions. Up to 30% of the variability is attributed to genetic polymorphisms.[99] Cytochrome P450 enzymes are major determinants of drug response. They are responsible for approximately 80% of Phase I drug metabolism, and 70% of drug clearance.[100] The human CYP supergene family includes 57 genes, 12 of

which are responsible for more than 75% of all drug oxidation reactions.[100] The CYP genes are highly polymorphic composed of large numbers of single-nucleotide polymorphisms (SNPs) and copy number variations. The most studied are genes of CYP2D6, 2C9, 2C8, 3A4, and 3A5 enzymes.[101–110] However, drug pharmacokinetics also depend on the renal excretion of the medications. MATE1, encoded by SLC47A1 gene, has been identified as a major efflux transporter involved in the renal excretion of many drugs including chloroquine.[91,111–115]

Pharmacogenomics Informing Chloroquine Malaria Pharmacotherapy

Individual variation in drug response is a critical challenge in effective drug pharmacotherapy. Both the nature of the drug, as well as the dose of the drug, are subjected to vary on an individual basis. Genetic polymorphisms in metabolizing enzymes influence the pharmacokinetics and drug response.[116]

Plasmodium vivax is the major cause of malaria disease outside Africa. The World Health Organization (WHO) recommends chloroquine as a component of the treatment protocol for uncomplicated *P. vivax* malaria.[117] Chloroquine is metabolized by the CYP450 isozymes 2C8, 3A4, 3A5 and 2D6. The CYP2C genes are located in a cluster on chromosome 10q24, organized as Cent-CYP2C18-CYP2C19-CYP2C9-CYP2C8-Tel.[118] The CYP2C8 gene is approximately 30 kb in size and includes nine exons.[119] CYP2C8 is the most divergent with respect to its protein sequence. Interindividual variability in chloroquine efficacy was previously reported in Africa and Asia and attributed to: *P. vivax* resistance to chloroquine, noncompliance, suboptimum dose and drug–drug interactions.[120] In a study reported in 2016, assessment of genetic polymorphisms in chloroquine metabolizing enzymes was identified as a need.[121] To that end, the investigators focused on a cohort consisting of 164 *P. vivax* malaria patients followed during malaria treatment from 2007 to 2009. The study reported for the first time the influence of the CYP2C8 gene on gametocyte clearance rate with patients undergoing chloroquine/primaquine malaria treatment. From baseline until the first day of treatment, wild-type CYP2C8 homozygous individuals achieved greater reduction in gametocytes as compared to individuals without this genotype. The results suggested that CYP2C8, CYP2C9 and CYP23A5 genetic variants influenced chloroquine malaria treatment.

Pharmacogenomics Informing Hydroxychloroquine Lupus Pharmacotherapy

Discoid lupus erythematosus is the most common form of cutaneous lupus.[122] Patients diagnosed with systemic lupus erythematosus and rheumatoid arthritis show a positive correlation between whole blood hydroxychloroquine levels and clinical response.[62] HCQ is metabolized to N-desethylhydroxychloroquine in the liver.[123] The reaction is mediated by CYP3A4, CYP2C8, CYP2D6 and CYP3A5 isoforms.[71,123] In a study reported by Lee et al, 194 systemic lupus erythematosus patients were genotyped for 4 SNPs in CYP3A4*18B, CYP2D6*10, CYP3A5*3.[124] The association of the respective genotypes with blood hydroxychloroquine and N-desethyl hydroxychloroquine was the focus of the investigation. The CYP2D6*10 allelic variants were found to be significantly associated with the N-desethylhydroxychloroquine/hydroxychloroquine ratio. The study demonstrated that this ratio is related to CYP2D6 polymorphisms in systemic lupus erythematosus patients treated with hydroxychloroquine.

A multicenter observational and pharmacogenetic study with 200 discoid lupus erythematosus patients treated with HCQ was reported by Wahie et al.[125] Thirty-nine percent of the patients failed to respond to hydroxychloroquine, or developed toxicity. The study showed a trend for CYP2C8 variants to be associated with better response.

Discussion

The PK and PD characteristics of chloroquine and hydroxychloroquine need to be evaluated in order to provide safe and effective COVID-19 therapy. The pharmacokinetics of chloroquine and hydroxychloroquine are similar. Oral absorption of the drugs is comparable with bioavailability values of 0.7–0.8.[47,48] But the two medications have significant variations in bioavailability between individuals.[49,50] Genetic polymorphism of CYP enzymes involved in the presystemic metabolism can explain at least in part the individual differences in the oral absorption of the drugs and may reflect the variations in blood and tissue concentrations of the drugs in COVID-19 patients.

Both CQ and HCQ have wide distribution to the body tissues. Plasma protein binding of the drugs varies between 50% and 60%.[47,64] CQ and HCQ are known to be enantioselective in their dispositions. Both medications have similar stereoselective patterns of protein binding. S (+)-isomers are more bound to plasma proteins than R(-)-isomers, suggesting that free plasma concentrations are higher for R(-)-forms.[45] Such differences in the plasma

protein binding can be responsible in part for the variations in therapeutic response and toxicity of the isomers in COVID-19 patients treated with CQ or HCQ, as only free drug can interact with receptors and produce therapeutic and/or side effects.

Chloroquine and hydroxychloroquine have long half-lives. The long half-life can be attributed to extensive tissue uptake rather than decreased elimination. Chloroquine is N-desethylated into two major metabolites largely by CYP3A4 and CYP2C8, while hydroxychloroquine metabolizes into three metabolites primarily by CYP3A4.[69,70] Metabolism of both drugs is stereoselective. The higher blood and plasma concentrations of (R)-forms confirm the stereoselective metabolism of the medications, where S-enantiomers metabolize faster than R-enantiomers.[74,81] As a result, S(+)-isomers have shorter half-life than R(-)-isomers. Similar doses of both medications produce large (11-fold) variations in the blood concentrations in patients with rheumatoid arthritis and in healthy volunteers.[47,64,82,83] Comparable differences in drug levels may be expected in COVID-19 patients. The variability can be explained by the stereoselective metabolism as well as genetic polymorphism of the P-450 enzymes involved in the biotransformation of the medications.

Individual variation in drug response is a critical challenge in effective drug pharmacotherapy. Up to 70% of individuals that receive a drug, exhibit lack of efficacy or adverse drug reactions, at least partially, due to genetic polymorphisms.[99] Gene polymorphisms influence metabolism as well as active transport.

CYP2D6 the most extensively studied CYP gene metabolizes approximately 25% of all drugs. Genetic polymorphisms resulting in increased CYP2D6 metabolic capabilities have been linked to decreased treatment response with tricyclic antidepressants, increased occurrences of respiratory depression and opioid toxicity.[101,102] CYP2C9 deficiency is related to bleeding complications with warfarin and other anticoagulants treatment. Fifty percent of interindividual variability in dose requirements is observed in concert with age, body surface area and polymorphisms in VKORC1.[103]

CYP2C8 is involved in the metabolism of many medications including non-steroidal anti-inflammatory drugs, thiazolidinediones, chemotherapy agents, chloroquine and hydroxychloroquine. CYP2C8 genotyping should be considered as a viable option in Africans and Europeans in which 19.2% and 17.2% of CYP2C8 alleles, respectively, exhibit reduced functionality.[104]

The CYP3A subfamily is the most abundant of the P-450 enzymes. CYP3A4 and CYP3A5 metabolize more than half of the marketed drugs.[105,106] The most common variant of CYP3A4 enzyme, CYP3A4*1B has been associated with reduced CYP3A4 activity.[107] The CYP3A4*1B allelic frequency varies among different ethnic groups, ranging from 0% in Chinese to 67% in African Americans.[108–110] CYP3A5 is polymorphically expressed in 10–20% in Caucasians, 33% in Japanese and 55% in African Americans.[109] The primary variant is CYP3A5*3, which has been associated with low CYP3A5 protein expression and reduced metabolic activity. The CYP3A5*3 allele frequency varies from approximately 50% in African Americans to 90% in Caucasians.[109] Other allelic variants have been reported for both CYP3A4 and CYP3A5. However, the variants occur at relatively low allelic frequencies and their functional significance has not been verified and validated.

Determination of CYP3A, CYP2C8 and CYP2D6 polymorphism and, therefore, activity is important to establish safe and efficient dosing of chloroquine and hydroxychloroquine for treatment of COVID-19 patients. A recent study reported for the first time the influence of the CYP2C8 gene on clearance in patients with chloroquine/primaquine therapy.[121] Wild-type CYP2C8 homozygous individuals achieved greater reduction in gametocytes as compared to individuals without this genotype.[121] Another study demonstrated CYP2D6 polymorphisms in systemic lupus erythematosus patients treated with hydroxychloroquine. CYP2D6*10 allelic variants were found to be significantly associated with altered metabolism of HCQ.[124] A study with 200 lupus erythematosus patients treated with hydroxychloroquine demonstrated 39% of the patients failed to respond to the therapy or developed toxicity.[125] Similar trend was observed in a recent clinical trial with COVID-patients treated with hydroxychloroquine.[43] Additionally, the study with 200 lupus erythematosus patients showed a trend for CYP2C8 variants to be associated with better response.[125] Overall, the results suggest that CYP2C8, CYP2D6 and CYP3A genetic polymorphisms may influence chloroquine and hydroxychloroquine pharmacokinetics and COVID-19 patients treated with the same dose of CQ or HCQ may exhibit lack of efficacy or adverse reactions. The variability in therapeutic response may require dose adjustment of CQ/HCQ in treatment of COVID-19 patients.

Urinary excretion is the primary route of elimination for chloroquine and hydroxychloroquine. The 50% of a CQ dose is recovered in the urine as unchanged drug, while only 16–21% of an HCQ dose is renally excreted as

unchanged drug.[16,47] However, a greater fraction of absorbed HCQ dose excretes in the feces compared to the fraction of CQ dose. The elimination half-lives of both medications are significantly longer in patients with chronic renal disease.[16,57] This finding recommends that the two drugs should be used with caution in patients with renal impairment, as kidney dysfunction may lead to greater drug retention and higher risk of adverse effects. Renal elimination of both compounds is stereoselective, (S)-isomers have a mean renal clearance approximately twice that of (R)-isomers.[81]

The main mechanism of renal elimination of the medications is tubular secretion.[57,90] However, the molecular mechanisms of the renal tubular secretion remain mostly unidentified. It was reported that chloroquine is a substrate and potent inhibitor of the MATE1 transporter.[91] Given the similarity in structure between CQ and HCQ, it is possible to propose that HCQ is also a substrate for MATE1. MATE1 is a proton-substrate antiporter expressed in the kidney and liver that facilitates the export of organic cations, such as metformin, paraquat, and oxaliplatin into urine and bile.[92] Genetic polymorphisms of MATE1 may alter the pharmacokinetics and pharmacodynamics of the medications, as drug transporters are key determinants of elimination of the drugs. MATE1, encoded by the SLC47A1 gene, has been identified as a major efflux transporter involved in the renal excretion of chloroquine.[91] Functional SNP of MATE1 (rs2289669 G>A) was associated with increased glucose-lowering activity of metformin through slowing renal excretion of the anti-diabetic drug.[111,112] The allele frequency ranges from 10.4% in African Americans to 49% in Mexican Americans.[113] Other SNPs may also alter transport activity of MATE1 and lead to changed elimination of the corresponding drugs.[114,115] Genetic polymorphisms of MATE1 can affect renal elimination of CQ and HCQ and, therefore, may require dose adjustment based on pharmacogenomic profiles of COVID-19 patients.

Indeed, the active transporters and CYP enzyme polymorphisms may explain the variations in blood concentrations, therapeutic responses and severity of adverse effects of chloroquine and hydroxychloroquine. Despite the evidence of the influence of genetic polymorphisms on the pharmacokinetics of chloroquine and hydroxychloroquine, no large pharmacogenomics studies have been conducted to provide guidance on the use, dosing, and duration of the therapy in COVID-19 patients.

Additionally, chloroquine and hydroxychloroquine are involved in several DDIs. Cimetidine and ketoconazole, CYP3A4 inhibitors increased serum concentrations of CQ.[71,84] Predictably, cimetidine and ketoconazole may also increase HCQ blood concentrations by inhibition metabolism of the drug. Co-administration of CQ and HCQ with moderate and strong CYP3A4 (boceprevir, cobicistat, azole anti-fungal agents, macrolide antibiotics, etc.), CYP2C8 (gemfibrozil, clopidogrel, deferasirox, teriflunomide) and MATE1 inhibitors may result in increased plasma concentrations, longer half-life, exaggerated therapeutic effect and the toxicity of chloroquine and hydroxychloroquine.[85] Significant drug interactions with chloroquine and hydroxychloroquine that should be avoided or require additional monitoring include digoxin, antiepileptics, antacids, cyclosporine, amiodarone, azithromycin, moxifloxacin, insulin and other antidiabetic agents, tamoxifen, and praziquantel.[32,33] With the currently known or potential DDIs, the use of chloroquine and hydroxychloroquine with other drug therapy requires consideration for patient safety in COVID-19 patients.

Conclusions

Limited pharmacogenomic studies have been performed investigating the inter-patient variability of chloroquine and hydroxychloroquine in both malaria and lupus patient populations. Moreover, data to support the use of hydroxychloroquine and chloroquine for COVID-19 are limited and inconclusive. The off-label use of chloroquine and hydroxychloroquine to treat COVID-19 must be used with caution given the toxicities: cardiac, retinal and cutaneous severe adverse effects. Well-designed randomized trials incorporating pharmacogenomics need to be performed in a timely manner to achieve safe and effective dosing and to reduce severity of adverse effects.

Abbreviations

CQ, chloroquine; DDI, drug–drug interaction; EC_{50}, 50% maximal effective concentration; FDA, Food and Drug Administration; HCQ, hydroxychloroquine; IBW, ideal body weight; MATE1, multidrug and toxin extrusion protein 1; PGx, pharmacogenomics; PK, pharmacokinetics; SNP, single nucleotide polymorphism.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Johns Hopkins University. OMIM gene map statistics; 2017. Available from: http://www.omim.org/statistics/geneMap. Accessed May 25, 2020.
2. National Institutes of Health. *Transforming Translational Research*. Rockville, MD; 2015.
3. Scannell J, Blanckley A, Boldon H, et al. Diagnosing the decline in pharmaceutical R&D efficiency. *Nat Rev Drug Discov*. 2012;11(3):191–200. doi:10.1038/nrd3681
4. Nosengo N. Can you teach old drugs new tricks? *Nature*. 2016;534(7607):314–316. doi:10.1038/534314a
5. Talevi A, Bellera CL. Challenges and opportunities with drug repurposing: finding strategies to find alternative uses of therapeutics. *Expert Opin Drug Discov*. 2020;15(4):397–401.
6. Talevi A. Drug repositioning: current approaches and their implications in the precision medicine era. *Expert Rev Precis Med Drug Dev*. 2018;3(1):49–61. doi:10.1080/23808993.2018.1424535
7. Hirsch HA, Iliopoulos D, Joshi A, et al. A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases. *Cancer Cell*. 2010;17(4):348–361. doi:10.1016/j.ccr.2010.01.022
8. Andersen PI, Ianevskia A, Lysvanda H, et al. Discovery and development of safe-in-man broad spectrum antiviral agents. *Int J Infect Dis*. 2020;93:268–276. doi:10.1016/j.ijid.2020.02.018
9. Drug.com. Aralen phosphate. the american society of health-system pharmacists. Available from: https://www.drugs.com/monograph/chloroquine-phosphate.html. Accessed June 15, 2020.
10. Drug.com. Hydroxychloroquine sulfate. The American society of health-system pharmacists. Available from: https://www.drugs.com/monograph/hydroxychloroquine-sulfate.html. Accessed June 29, 2020.
11. Li C, Zhu X, Ji X, et al. Chloroquine, FDA-approved drug, prevents Zika virus infection and its associated congenital microcephaly in mice. *EBioMedicine*. 2017;24:189–194.
12. Shiryaev SA, Mesci P, Pint A, et al. Repurposing of the anti-malaria drug chloroquine for Zika Virus treatment and prophylaxis. *Sci Rep*. 2017;7(1):15771. doi:10.1038/s41598-017-15467-6
13. Plantone D, Koudriavtseva T. Current and future use of chloroquine and hydroxychloroquine in infectious, immune, neoplastic, and neurological diseases: a mini-review. *Clin Drug Investig*. 2018;38(8):653–671.
14. Coronado LM, Nadovich CT, Spadafora C. Malarial hemozoin: from target to tool. *Biochim Biophys Acta*. 2014;1840(6):2032–2041. doi:10.1016/j.bbagen.2014.02.009
15. Fox RI. Mechanism of action of hydroxychloroquine as an antirheumatic drug. *Semin Arthritis Rheum*. 1993;23(2 Suppl 1):82–91. doi:10.1016/S0049-0172(10)80012-5
16. Ducharme J, Farinotti R. Clinical pharmacokinetics and metabolism of chloroquine. Focus on recent advancements. *Clin Pharmacokinet*. 1996;31(4):257–274. doi:10.2165/00003088-199631040-00003
17. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res*. 2020;30(3):269–271. doi:10.1038/s41422-020-0282-0
18. Vincent MJ, Bergeron E, Benjannet S, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol J*. 2005;2(1):69. doi:10.1186/1743-422X-2-69
19. Yao X, Ye F, Zhang M, et al. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis*. 2020;71(15):732–739. doi:10.1093/cid/ciaa237
20. Weniger H. Review of side effects and toxicity of chloroquine. *Bull World Health*. 1979;79:906.
21. McChesney EW. Animal toxicity and pharmacokinetics of hydroxychloroquine sulfate. *Am J Med*. 1983;75(1A):11–18. doi:10.1016/0002-9343(83)91265-2
22. Liu J, Cao R, Xu M, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. *Cell Discov*. 2020;6(1):16. doi:10.1038/s41421-020-0156-0
23. Tönnesmann E, Kandolf R, Lewalter T. Chloroquine cardiomyopathy - a review of the literature. *Immunopharmacol Immunotoxicol*. 2013;35(3):434–442. doi:10.3109/08923973.2013.780078
24. Chorin E, Dai M, Shulman E, et al. The QT interval in patients with SARS-CoV-2 infection treated with hydroxychloroquine/azithromycin. *medRxiv*. 2020;04(02):20047050.
25. Mzayek F, Deng H, Mather FJ, et al. Randomized dose-ranging controlled trial of AQ-13, a candidate antimalarial, and chloroquine in healthy volunteers. *PLoS Clin Trials*. 2007;2(1):e6. doi:10.1371/journal.pctr.0020006
26. Morgan ND, Patel SV, Dvorkina O. Suspected hydroxychloroquine-associated QTinterval prolongation in a patient with systemic lupus erythematosus. *J Clin Rheumatol*. 2013;19(5):286–288. doi:10.1097/RHU.0b013e31829d5e50
27. O'Laughlin JP, Mehta PH, Wong BC. Life threatening severe QTc prolongation in patient with systemic lupus erythematosus due to hydroxychloroquine. *Case Rep Cardiol*. 2016;2016:4626279.
28. Flach AJ. Improving the risk-benefit relationship and informed consent for patients treated with hydroxychloroquine. *Trans Am Ophthalmol Soc*. 2007;105:191–197.
29. Marmor MF, Kellner U, Lai TY, et al. American academy of ophthalmology. revised recommendations on screening for chloroquine and hydroxychloroquine retinopathy. *Ophthalmology*. 2011;118(2):415–422. doi:10.1016/j.ophtha.2010.11.017
30. Schroeder RL, Gerber JP. Chloroquine and hydroxychloroquine binding to melanin: some possible consequences for pathologies. *Toxicol Rep*. 2014;1:963–968. doi:10.1016/j.toxrep.2014.10.019
31. Srinivasa A, Tosounidou S, Gordon C. Increased incidence of gastrointestinal side effects in patients taking hydroxychloroquine: a brand-related issue? *J Rheumatol*. 2017;44(3):398. doi:10.3899/jrheum.161063
32. FDA. EUA chloroquine phosphate health care provider fact sheet. Available from: https://www.fda.gov/media/136535/download. Accessed July 14, 2020.
33. FDA. EUA hydroxychloroquine sulfate health care provider fact sheet. Available from: https://www.fda.gov/media/136537/download. Accessed April 28, 2020.
34. Savarino A, Boelaert JR, Cassone A, et al. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis*. 2003;3(11):722–727. doi:10.1016/S1473-3099(03)00806-5
35. Savarino A, Di Trani L, Donatelli I, et al. New insights into the antiviral effects of chloroquine. *Lancet Infect Dis*. 2006;6(2):67–69. doi:10.1016/S1473-3099(06)70361-9
36. Biot C, Daher W, Chavain N, et al. Design and synthesis of hydroxyferroquine derivatives with antimalarial and antiviral activities. *J Med Chem*. 2006;49(9):2845–2849. doi:10.1021/jm0601856
37. Colson P, Rolain JM, Lagier JC, et al. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. *Int J Antimicrob Agents*. 2020;55(4):105932. doi:10.1016/j.ijantimicag.2020.105932
38. Cortegiani A, Ingoglia G, Ippolito M, et al. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. *J Crit Care*. 2020;57:279–283.
39. Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends*. 2020;14(1):72–73. doi:10.5582/bst.2020.01047

40. Gautret P, Lagier JC, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents*. 2020;56(1):105949. doi:10.1016/j.ijantimicag.2020.105949
41. Molina JM, Delaugerre C, Le Goff J, et al. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. *Med Mal Infect*. 2020;50(4):384. doi:10.1016/j.medmal.2020.03.006
42. Chen J, Liu D, Liu L, et al. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). *J Zhejiang Univ Med Sci*. 2020;49(2):215–219.
43. Magagnoli J, Narendran S, Pereira F, et al. Outcomes of hydroxychloroquine usage in United States veterans hospitalized with covid-19.medRxiv; 2020. Available from: https://www.medrxiv.org/content/10.1101/2020.04.16.20065920v2. Accessed May 28, 2020.
44. FDA. Coronavirus (COVID-19) update: FDA reiterates importance of close patient supervision for 'off-label' use of antimalarial drugs to mitigate known risks, including heart rhythm problems; 2020. Available from: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-reiterates-importance-close-patient-supervision-labeluse?utm_campaign=042420_PR_FDA%20Notes%20Importance%20of%20Patient%20Supervision%20of%20Antimalarial%20Drug%20Use&utm_medium=email&utm_source=Eloqua. Accessed May 8, 2020.
45. Iredale J, Fieger H, Wainer IW. Determination of the stereoisomers of hydroxychloroquine and its major metabolites in plasma and urine following a single oral administration of racemic hydroxychloroquine. *Semin Arthritis Rheum*. 1993;23(2 Suppl 1):74–81. doi:10.1016/S0049-0172(10)80011-3
46. Mackenzie AH. Pharmacologic actions of 4-aminoquinoline compounds. *Am J Med*. 1983;75(1A):5–10. doi:10.1016/0002-9343(83)91264-0
47. Furst DE. Pharmacokinetics of hydroxychloroquine and chloroquine during treatment of rheumatic diseases. *Lupus*. 1996;5 (Suppl 1):S11–S15. doi:10.1177/096120339600500104l
48. Titus EO. Recent developments in the understanding of the pharmacokinetics and mechanism of action of chloroquine. *Ther Drug Monit*. 1989;11(4):369–379. doi:10.1097/00007691-198907000-00001
49. Tett SE. Clinical pharmacokinetics of slow-acting antirheumatic drugs. *Clin Pharmacokinet*. 1993;25(5):392–407. doi:10.2165/00003088-199325050-00005
50. Bothwell B, Furst DE. Hydroxychloroquine. In: Day RO, Fürst DE, van Riel PLCM, Bresnihan B, editors. *Antirheumatic Therapy: Actions and Outcomes. Progress in Inflammation Research*. Birkhäuser Basel; 2005:81–92.
51. Tett SE, Cutler DJ, Day RO, Brown KF. Bioavailability of hydroxychloroquine tablets in healthy volunteers. *Br J Clin Pharmacol*. 1989;27(6):771–779. doi:10.1111/j.1365-2125.1989.tb03439.x
52. Tett S, Day R, Cutler D. Hydroxychloroquine relative bioavailability: within subject reproducibility. *Br J Clin Pharmacol*. 1996;41(3):244–246. doi:10.1111/j.1365-2125.1996.tb00190.x
53. Tett S, Cutler D, Day R. Antimalarials in rheumatic diseases. *Baillieres Clin Rheumatol*. 1990;4(3):467–489. doi:10.1016/S0950-3579(05)80004-4
54. Marquardt K, Albertson TE. Treatment of hydroxychloroquine overdose. *Am J Emerg Med*. 2001;19(5):420–424. doi:10.1053/ajem.2001.25774
55. Miller A, Harrell E, Ye L, et al. Pharmacokinetic interactions and safety evaluations of coadministered tafenoquine and chloroquine in healthy subjects. *Br J Clin Pharmacol*. 2013;76(6):858–867. doi:10.1111/bcp.12160
56. Salako LA, Aderounmu AF, Walker O. Influence of route of administration on the pharmaco-kinetics of chloroquine and desethylchloroquine. *Bull World Health Organ*. 1987;65 (1):47–50.
57. Gustafsson LL, Walker O, Alvan G, et al. Disposition of chloroquine in man after single intravenous and oral doses. *Br J Clin Pharmacol*. 1983;15(4):471–479. doi:10.1111/j.1365-2125.1983.tb01532.x
58. Olafuyi O, Badhan RKS. Dose optimization of chloroquine by pharmacokinetic modeling during pregnancy for the treatment of Zika virus infection. *J Pharm Sci*. 2019;108(1):661–673. doi:10.1016/j.xphs.2018.10.056
59. Popert AJ. Choloroquine: a review. *Rheumatology*. 1976;15 (3):235–238. doi:10.1093/rheumatology/15.3.235
60. Tanaka E, Taniguchi A, Urano W, et al. Pharmacogenetics of disease-modifying anti-rheumatic drugs. *Best Pract Res Clin Rheumatol*. 2004;18(2):233–247. doi:10.1016/j.berh.2004.02.006
61. Rainsford KD, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. *Inflammopharmacology*. 2015;23(5):231–269.
62. Tett SE, Day RO, Cutler DJ. Concentration-effect relationship of hydroxychloroquine in rheumatoid arthritis–a cross sectional study. *J Rheumatol*. 1993;20(11):1874–1879.
63. Munster T, Gibbs JP, Shen D, et al. Hydroxychloroquine concentration-response relationships in patients with rheumatoid arthritis. *Arthritis Rheum*. 2002;46(6):1460–1469. doi:10.1002/art.10307
64. Lim HS, Im JS, Cho JY, et al. Pharmacokinetics of hydroxychloroquine and its clinical implications in chemoprophylaxis against malaria caused by Plasmodium vivax. *Antimicrob Agents Chemother*. 2009;53(4):1468–1475. doi:10.1128/AAC.00339-08
65. Walker O, Birkett DJ, Alván G, et al. Characterization of chloroquine plasma protein binding in man. *Br J Clin Pharmacol*. 1983;15(3):375–377. doi:10.1111/j.1365-2125.1983.tb01513.x
66. Ofori-Adjei D, Ericsson O, Lindström B, Sjöqvist F. Protein binding of chloroquine enantiomers and desethylchloroquine. *Br J Clin Pharmacol*. 1986;22(3):356–358. doi:10.1111/j.1365-2125.1986.tb02900.x
67. Ducharme J, Wainer IW, Parenteau HI, Rodman JH. Stereoselective distribution of hydroxychloroquine in the rabbit following single and multiple oral doses of the racemate and the separate enantiomers. *Chirality*. 1994;6(4):337–346. doi:10.1002/chir.530060418
68. Rollo IM. Drugs used in the chemotherapy of malaria. In: Gilman AG, Goodman LS, Gilman A, editors. *The Pharmacological Basis of Therapeutics*. New York: Macmillan; 1980:1038–1060.
69. Kalia S, Dutz JP. New concepts in antimalarial use and mode of action in dermatology. *Dermatol Ther*. 2007;20(4):160–174. doi:10.1111/j.1529-8019.2007.00131.x
70. Rengelshausen J, Burhenne J, Fröhlich M, et al. Pharmacokinetic interaction of chloroquine and methylene blue combination against malaria. *Eur J Clin Pharmacol*. 2004;60(10):709–715. doi:10.1007/s00228-004-0818-0
71. Kim KA, Park JY, Lee JS, Lim S. Cytochrome P450 2C8 and CYP3A4/5 are involved in chloroquine metabolism in human liver microsomes. *Arch Pharm Res*. 2003;26(8):631–637. doi:10.1007/BF02976712
72. Kaewkhao K, Chotivanich K, Winterberg M, et al. High sensitivity methods to quantify chloroquine and its metabolite in human blood samples using LC-MS/MS. *Bioanalysis*. 2019;11 (5):333–347. doi:10.4155/bio-2018-0202
73. Projean D, Baune B, Farinotti R, et al. In vitro metabolism of chloroquine: identification of CYP2C8, CYP3A4, and CYP2D6 as the main isoforms catalyzing N-desethylchloroquine formation. *Drug Metab Dispos*. 2003;31(6):748–754. doi:10.1124/dmd.31.6.748

74. Gustafsson LL, Nordmark B, Hermansson J. The pharmacokinetics of (+)- and (-)-chloroquine in patients with rheumatoid arthritis. Proceedings of the 3rd World Congress on Clinical Pharmacology and Therapeutics; 1986; Stockholm, Sweden.
75. Augustijns P, Verbeke N. Stereoselectivity in the disposition of chloroquine and desethylchloroquine in rabbits. *Arzneimittelforschung*. 1992;42(6):825–828.
76. Ducharme J, Fieger H, Ducharme MP, et al. Enantioselective disposition of hydroxychloroquine after a single oral dose of the racemate to healthy subjects. *Br J Clin Pharmacol*. 1995;40(2):127–133. doi:10.1111/j.1365-2125.1995.tb05768.x
77. Collins KP, Jackson KM, Gustafson DL. Hydroxychloroquine: a physiologically-based pharmacokinetic model in the context of cancer-related autophagy modulation. *J Pharmacol Exp Ther*. 2018;365(3):447–459. doi:10.1124/jpet.117.245639
78. Cardoso CD, Bonato PS. Enantioselective analysis of the metabolites of hydroxychloroquine and application to an in vitro metabolic study. *J Pharm Biomed Anal*. 2005;37(4):703–708. doi:10.1016/j.jpba.2004.11.048
79. Cardoso CD, Jabor VP, Bonato PS. Capillary electrophoretic chiral separation of hydroxychloroquine and its metabolites in the microsomal fraction of liver homogenates. *Electrophoresis*. 2006;27(5–6):1248–1254. doi:10.1002/elps.200500752
80. Cardoso CD, Bonato PS. Enantioselective metabolism of hydroxychloroquine employing rats and mice hepatic microsomes. *Braz J Pharm Sci*. 2009;45(4):4. doi:10.1590/S1984-82502009000400008
81. McLachlan AJ, Tett SE, Cutler DJ, Day RO. Disposition of the enantiomers of hydroxychloroquine in patients with rheumatoid arthritis following multiple doses of the racemate. *Br J Clin Pharmacol*. 1993;36(1):78–81. doi:10.1111/j.1365-2125.1993.tb05897.x
82. Tett SE, McLachlan AJ, Cutler DJ, Day RO. Pharmacokinetics and pharmacodynamics of hydroxychloroquine enantiomers in patients with rheumatoid arthritis receiving multiple doses of racemate. *Chirality*. 1994;6(4):355–359. doi:10.1002/chir.530060420
83. Furst DE, Lindsley H, Baethge B, et al. Dose-loading with hydroxychloroquine improves the rate of response in early, active rheumatoid arthritis: a randomized, double-blind six-week trial with eighteen-week extension. *Arthritis Rheum*. 1999;42(2):357–365. doi:10.1002/1529-0131(199902)42:2<357::AID-ANR19>3.0.CO;2-J
84. Ette EI, Brown-Awala EA, Essien EE. Chloroquine elimination in humans: effect of low-dose cimetidine. *J Clin Pharmacol*. 1987;27(10):813–816. doi:10.1002/j.1552-4604.1987.tb03002.x
85. FDA. Drug development and drug interactions. Available from: https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers#table3-2. Accessed July 3, 2020.
86. McChesney EW, Fasco MJ, Banks WF Jr. The metabolism of chloroquine in man during and after repeated oral dosage. *J Pharmacol Exp Ther*. 1967;158(2):323–331.
87. Jailer JW, Rosenfeld M, Shannon JA. The influence of orally administered alkali and acid on the renal excretion of quinacrine, chloroquine and santoquine. *J Clin Invest*. 1947;26(6):1168–1172. doi:10.1172/JCI101909
88. Browning DJ. Pharmacology of Chloroquine and Hydroxychloroquine. In: *Hydroxychloroquine and Chloroquine Retinopathy*. New York, NY: Springer; 2014:35–63.
89. Drug Bank. Hydroxychloroquine. Available from: https://www.drugbank.ca/drugs/DB01611. Accessed May 30, 2020.
90. Walker O, Salako LA, Alván G, et al. The disposition of chloroquine in healthy Nigerians after single intravenous and oral doses. *Br J Clin Pharmacol*. 1987;23(3):295–301. doi:10.1111/j.1365-2125.1987.tb03048.x
91. Müller F, König J, Glaeser H, et al. Molecular mechanism of renal tubular secretion of the antimalarial drug chloroquine. *Antimicrob Agents Chemother*. 2011;55(7):3091–3098.
92. Otsuka M, Matsumoto T, Morimoto R, et al. A human transporter protein that mediates the final excretion step for toxic organic cations. *Proc Natl Acad Sci U S A*. 2005;102(50):17923–17928.
93. Seideman P, Albertioni F, Beck O, et al. Chloroquine reduces the bioavailability of methotrexate in patients with rheumatoid arthritis. A possible mechanism of reduced hepatotoxicity. *Arthritis Rheum*. 1994;37(6):830–833. doi:10.1002/art.1780370609
94. Xu C, Zhu L, Chan T, et al. Chloroquine and hydroxychloroquine are novel inhibitors of human organic anion transporting polypeptide 1A2. *J Pharm Sci*. 2016;105(2):884–890. doi:10.1002/jps.24663
95. Senarathna SM, Page-Sharp M, Crowe A. The interactions of P-glycoprotein with antimalarial drugs, including substrate affinity, inhibition and regulation. *PLoS One*. 2016;11(4):e0152677. doi:10.1371/journal.pone.0152677
96. Nampoory MR, Nessim J, Gupta RK, Johny KV. Drug interaction of chloroquine with ciclosporin. *Nephron*. 1992;62(1):108–109.
97. Finielz P, Gendoo Z, Chuet C, Guiserix J. Interaction between cyclosporin and chloroquine. *Nephron*. 1993;65(2):333. doi:10.1159/000187506
98. Somer M, Kallio J, Pesonen U, et al. Influence of hydroxychloroquine on the bioavailability of oral metoprolol. *Br J Clin Pharmacol*. 2000;49(6):549–554. doi:10.1046/j.1365-2125.2000.00197.x
99. Eichelbaum M, Ingelman-Sundberg M, Evans WE. Pharmacogenomics and individualized drug therapy. *Annu Rev Med*. 2006;57(1):119–137. doi:10.1146/annurev.med.56.082103.104724
100. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science*. 1999;286(5439):487–491. doi:10.1126/science.286.5439.487
101. Kawanishi C, Lundgren S, Agren H, Bertilsson L. Increased incidence of CYP2D6 gene duplication in patients with persistent mood disorders: ultrarapid metabolism of antidepressants as a cause of nonresponse. A pilot study. *Eur J Clin Pharmacol*. 2004;59(11):803-807.
102. Stamer UM, Stüber F, Muders T, Musshoff F. Respiratory depression with tramadol in a patient with renal impairment and CYP2D6 gene duplication. *Anesth Analg*. 2008;107(3):926–929. doi:10.1213/ane.0b013e31817b796e
103. Johnson JA, Gong L, Whirl-Carrillo M, et al. Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. *Clin Pharmacol Ther*. 2011;90(4):625–629. doi:10.1038/clpt.2011.185
104. Zhou Y, Ingelman-Sundberg M, Lauschke VM. Worldwide distribution of cytochrome P450 alleles: a meta-analysis of population-scale sequencing projects. *Clin Pharmacol Ther*. 2017;102(4):688-700. doi:10.1002/cpt.690
105. Rendic S. Summary of information on human CYP enzymes: human P450 metabolism data. *Drug Metab Rev*. 2002;34(1–2):83–448.
106. Berno G, Zaccarelli M, Gori C, et al. Analysis of single-nucleotide polymorphisms (SNPs) in human CYP3A4 and CYP3A5 genes: potential implications for the metabolism of HIV drugs. *BMC Med Genet*. 2014;15(1):76. doi:10.1186/1471-2350-15-76
107. Rebbeck TR, Jaffe JM, Walker AH, et al. Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. *J Natl Cancer Inst*. 1998;90(16):1225–1229.
108. Hsieh KP, Lin YY, Cheng CL, et al. Novel mutations of CYP3A4 in Chinese. *Drug Metab Dispos*. 2001;29(3):268–273.
109. Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev*. 2002;54(10):1271–1294. doi:10.1016/S0169-409X(02)00066-2

110. Paris PL, Kupelian PA, Hall JM, et al. Association between a CYP3A4 genetic variant and clinical presentation in African-American prostate cancer patients. *Cancer Epidemiol Biomarkers Prev*. 1999;8(10):901–905.
111. Becker ML, Visser LE, van Schaik RH, et al. Interaction between polymorphisms in the OCT1 and MATE1 transporter and metformin response. *Pharmacogenet Genomics*. 2010;20(1):38–44. doi:10.1097/FPC.0b013e328333bb11
112. He R, Zhang D, Lu W, et al. SLC47A1 gene rs2289669 G>A variants enhance the glucose-lowering effect of metformin via delaying its excretion in Chinese type 2 diabetes patients. *Diabetes Res Clin Pract*. 2015;109(1):57–63. doi:10.1016/j.diabres.2015.05.003
113. Raj GM, Mathaiyan J, Wyawahare M, et al. Genetic polymorphisms of multidrug and toxin extrusion proteins (MATE1 and MATE2) in South Indian population. *Bioimpacts*. 2017;7(1):25–30. doi:10.15171/bi.2017.04
114. Kajiwara M, Terada T, Ogasawara K, et al. Identification of multidrug and toxin extrusion (MATE1 and MATE2-K) variants with complete loss of transport activity. *J Hum Genet*. 2009;54(1):40–46. doi:10.1038/jhg.2008.1
115. Chen Y, Teranishi K, Li S, et al. Genetic variants in multidrug and toxic compound extrusion-1, hMATE1, alter transport function. *Pharmacogenomics J*. 2009;9(2):127–136. doi:10.1038/tpj.2008.19
116. Meyer U. Pharmacogenetics – five decades of therapeutic lessons from genetic diversity. *Nat Rev Genet*. 2004;5(9):669–676. doi:10.1038/nrg1428
117. Ferreira MU, Castro MC. Challenges for malaria elimination in Brazil. *Malar J*. 2016;15:284.
118. Gray IC, Nobile C, Muresu R, et al. A 2.4-megabase physical map spanning the CYP2C gene cluster on chromosome 10q24. *Genomics*. 1995;28(2):328–332. doi:10.1006/geno.1995.1149
119. Klose TS, Blaisdell JA, Goldstein JA. Gene structure of CYP2C8 and extrahepatic distribution of the human CYP2Cs. *J Biochem Mol Toxicol*. 1999;13(6):289–295. doi:10.1002/(SICI)1099-0461-(1999)13:6<289::AID-JBT1>3.0.CO;2-N
120. Baird JK. Effectiveness of antimalarial drugs. *N Engl J Med*. 2005;352(15):1565–1577. doi:10.1056/NEJMra043207
121. Sortica VA, Lindenau JD, Cunha MG, et al. The effect of SNPs in CYP450 in chloroquine/primaquine Plasmodium vivax malaria treatment. *Pharmacogenomics*. 2016;17(17):1903–1911. doi:10.2217/pgs-2016-0131
122. Tebbe B, Orfanos CE. Epidemiology and socioeconomic impact of skin disease in lupus erythematosus. *Lupus*. 1997;6(2):96–104. doi:10.1177/096120339700600204
123. Davila I, Ranganathan P. Pharmacogenetics: implications for therapy in rheumatic diseases. *Nat Rev Rheumatol*. 2011;7(9):537–550. doi:10.1038/nrrheum.2011.117
124. Lee JY, Vinayagamoorthy N, Han K, et al. Association of polymorphisms of cytochrome P450 2D6 with blood hydroxychloroquine levels in patients with systemic lupus erythematosus. *Arthritis Rheumatol*. 2016;68(1):184–190. doi:10.1002/art.39402
125. Wahie S, Daly AK, Cordell HJ, et al. Clinical and pharmacogenetic influences on response to hydroxychloroquine in discoid lupus erythematosus: a retrospective cohort study. *J Invest Dermatol*. 2011;131(10):1981–1986. doi:10.1038/jid.2011.167
126. Schwartz RA, Janniger CK. Generalized pustular figurate erythema: a newly delineated severe cutaneous drug reaction linked with hydroxychloroquine. *Dermatol Ther*. 2020;33(3):e13380. doi:10.1111/dth.13380