CLINICAL & FORENSIC

Toxicology News

ISSN 2326-3253

September 2020

An AACC/CAP Educational Newsletter for Toxicology Laboratories

Hydroxychloroquine and Chloroquine: Old Drugs Repurposed as Treatments for COVID-19

By Matthew D. Krasowski, MD, PhD

Introduction

The coronavirus disease 2019 (COVID-19) pandemic has had a substantial impact on global public health, economy, and travel (1). This has spawned an investigation into medications to treat and prevent complications resulting from infection by the causative viral pathogen SARS-CoV-2 (2). Extensive research has been undertaken to evaluate the efficacy of existing antiviral and immunomodulatory medications against COVID-19, pending development of novel therapies including vaccines. Examples of antiviral medications being evaluated for effectiveness against SARS-CoV-2 include remdesivir (currently a first-tier medication for the treatment of moderate to severe infections) and ribavirin. Dexamethasone, a glucocorticoid, has shown promise in a clinical trial in hospitalized COVID-19 patients and is hypothesized to provide benefit by modulating inflammation-mediated lung injury from SARS-CoV-2 (3). Two other drugs, hydroxychloroquine (HCQ) and chloroquine (CQ), have also gained substantial media and healthcare attention following reports of their efficacy against SARS-CoV-2 in early *in vitro* and small, uncontrolled clinical trials (4, 5). Subsequent larger studies, including randomized control trials, have shown mixed efficacy against COVID-19 infection, and the actual effectiveness of these medications for SARS-CoV-2 is still controversial (2, 5-8). Two of the larger trials have evaluated HCQ with or without azithromycin (5, 6).

The media and medical attention on CQ and HCQ as therapies for COVID-19 led to dramatically increased demand for both medications, resulting in the United States Food and Drug Administration adding both medications to drug shortage lists by the end of March 2020 (9). Multiple states reported inappropriate prescribing and hoarding of CQ and HCQ, including healthcare providers prescribing for themselves and family members (10). Short supplies of the medications created issues with patients taking these drugs for established indications such as rheumatoid arthritis or systemic lupus erythematosus. In addition, reports of toxicity from non-prescription sources of HCQ and CQ have emerged, including an Arizona man who died on March 23, 2020, after ingesting chloroquine phosphate that was formulated as an aquarium cleaner (11).

This report will discuss what is known about the pharmacology and toxicity of HCQ and CQ. These medications have many decades of clinical use, and there is substantial literature on the acute and chronic toxicity of these drugs. The use of these drugs for COVID-19 presents some unique challenges, as will be discussed.

History and Clinical Use of Chloroquine and Hydroxychloroquine

CQ and HCQ are synthetic quinine derivatives developed from compounds found in the bark of the cinchona (Family: Rubiaceae), a tree that contains quinine and some other alkaloids with antimalarial activity. Both drugs were first used therapeutically as antimalarials and represented a major medical advance in the treatment of malaria (12). CQ was discovered in 1934 and, along with some other quinine derivatives, has a long history of use for the treatment of malaria and amebiasis. CQ is very inexpensive and is used throughout the world. CQ is on the World Health Organization (WHO) List of Essential Medications for the treatment of malaria

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and amebiasis (13). Unfortunately, in recent years the rate of resistance of malaria parasites to CQ has increased, prompting a shift to other medications for malaria (12).

HCQ was initially synthesized from CQ in 1946 as an alternative antimalarial with lower toxicity, as the toxic adverse effects of CQ were already well described by the 1940s (14, 15). The two drugs are structurally very close to one another and differ by only a single hydroxyl group (see Figure 1 for chemical structures). While HCQ has also been used historically for malaria treatment and prophylaxis, the more common current clinical use is to treat autoimmune conditions such as systemic lupus erythematosus and rheumatoid arthritis (16, 17). In general, HCQ demonstrates less toxicity and more anti-inflammatory effects than CQ. On the WHO List of Essential Medications, CQ is listed as an essential disease-modifying medication for rheumatic diseases, with HCQ listed as complementary for this indication (13). For this type of indication, CQ is much lower in cost, but does have more risk of adverse effects compared to HCQ. Thus, HCQ is the more common treatment compared to CQ for autoimmune diseases such as rheumatoid arthritis

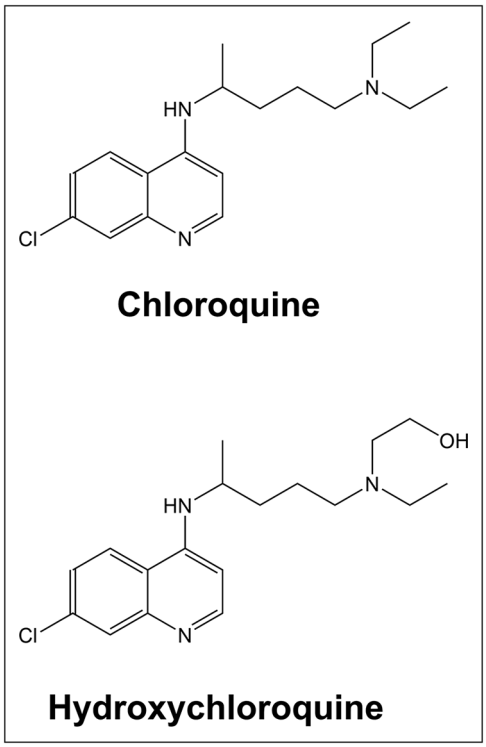


Figure 1. Chemical structures of chloroquine and hydroxychloroquine.

in developed countries, while CQ would be more common in resource-limited settings.

Antiviral properties of CQ and HCQ have also been described (18). The presumed mechanism of antiviral action for CQ and HCQ is by conversion to aminoquinolones that raise the intracellular pH in lysosomes and other intracellular acidic compartments and organelles. Viral replication depends on a stable acidic pH in endosomes, Golgi complex, and lysosomes. CQ and HCQ may additionally help in the treatment of COVID-19 by reducing inflammatory processes in the production of pro-inflammatory mediators such as cytokines.

Pharmacokinetic Properties of Chloroquine and Hydroxychloroquine

CQ and HCQ are absorbed rapidly and completely following oral administration, with peak whole blood levels achieved 1 to 3 h after ingestion (16, 17). When ingested in overdose, the peak drug concentrations correlate most closely with the onset of symptoms and the risk of mortality. Both drugs are metabolized by the cytochrome P450 (CYP) enzymes, leading to potential drug-drug interactions with drugs that inhibit CYP2C8 and/or CYP3A4/5 such as clopidogrel, gemfibrozil, antifungal azoles, ciprofloxacin, diltiazem, erythromycin, and verapamil (19). Concomitant use of CQ or HCQ with CYP inhibitors can lead to elevated blood concentrations and a higher risk of toxicity. CQ and HCQ both have large apparent volumes of distribution due to extensive binding to tissues. The terminal half-life of CQ is 5−12 days and 30−60 days for HCQ. In acute overdose, severe symptoms typically appear within the first several hours, but patients may exhibit toxicity for days or weeks (14, 20). Both drugs are transported across the placenta and also distribute into breast milk; however, both drugs are considered safe in pregnancy and for breastfeeding as long-term studies have not demonstrated adverse effects on fetuses or breastfeeding infants.

Acute Toxicity of Chloroquine and Hydroxychloroquine

The risk of acute toxicity from typically prescribed doses of HCQ and CQ is low (14, 20). Doses used for malaria prophylaxis in adults are usually between 300 and 400 mg/day. Maintenance dosing in adults for HCQ for autoimmune diseases is approximately 400 mg/day. For acute treatment of malaria, initial doses of CQ or HCG may be as high as 800 to 1000 mg/day, but then tapering to lower maintenance doses (5, 6). Protocols for treating COVID-19 may start with doses as high as 800 mg orally and then transition to doses between 400 and 600 mg (2, 5-7). This type of dosing strategy is therefore similar to those used for acute malaria

Table 1. Toxic Effects of Chloroquine (CQ) and Hydroxychloroquine (HCQ)

Target	Acute toxicity symptoms	Acute toxicity risk factors (1)	Chronic toxicity symptoms	Chronic toxicity risk factors
Eye	Uncommon, symptoms such as blurred vision related to the nervous system	Other drugs affecting vision	Retinal injury	Lifetime exposure to CQ and HCQ (especially >7 years)
Heart/cardiovascular	Arrhythmias, hypotension, hypokalemia	Genetic arrhythmia syndromes (e.g., long QT syndrome), heart failure	Cardiomyopathy	Prior history of cardiomyopathy, congestive heart failure, lifetime exposure to CQ and HCQ
Nervous system	Generalized weakness, vertigo, seizures (CQ only)	Other central nervous system acting drugs, especially depressants	Neuromyopathy	Lifetime exposure to CQ and HCQ

[1] Common risks for acute toxicity include high peak drug levels of CQ or HCQ and drug-drug interactions involving cytochrome P450 inhibitors

treatment. Consequently, there is a higher risk for acute toxicity at the onset of treatment regimens using these higher doses. There is also the possibility that people using this drug outside of prescribed medical regimens may ingest significantly higher doses.

Much of the literature on the acute toxicity of CQ and HCQ resides in case reports or case series of inadvertent or intentional overdoses of these drugs (14, 15, 20-22). Similar to other structural derivatives of quinine, CQ and HCQ exhibit cardiovascular toxicity through blockade of voltage-dependent potassium and sodium channels. These effects on cardiac ion channels underlie prolongation of the QRS and QT intervals on the electrocardiogram (ECG) that can be seen with CQ or HCQ therapy. This places patients at higher risk for Torsades de Pointes or other serious arrhythmias. Both drugs can also produce hypotension and cardiogenic shock by direct cardio-depressant effects. Hypokalemia is also common in acute CQ overdoses and likely due to intracellular shifts in potassium (21). Literature reports of HCQ overdoses are less common than for the more toxic CQ, although published case reports of HCQ toxicity can be found as early as 1960 (23). Seizures have been well-documented following acute CQ overdose; in contrast, there have been no reports of seizures following an overdose of HCQ (22). There have been reports of neurologic symptoms such as blurred vision, generalized weakness, and vertigo as a result of acute CQ or HCQ overdose (14, 15, 20-22). Table 1 summarizes symptoms of acute toxicity and risk factors.

Treatment of CQ and HCQ overdose focuses on supportive care including mechanical ventilation and vasopressor support (14, 15, 20, 22). Other treatment options include potassium replacement, lipid emulsion, sodium bicarbonate, and diltiazem. Potassium repletion should be done cautiously, given the risk of rebound hyperkalemia as toxicity resolves and potassium redistributes. There is currently no antidote for overdoses of HCQ or CQ.

Measurement of blood or other body fluid concentrations of HCQ and CQ is not commonly performed clinically (14, 15, 20, 22). This type of testing is only offered by a small number of specialized toxicology reference laboratories (24). However, it is worth noting that an acute overdose of either drug can lead to very high concentrations in urine. There are reports of interference with various types of urine testing following an acute overdose of HCQ and CQ, including assays for microalbumin, drug abuse screening, and total protein (24, 25). These interferences could potentially produce diagnostic confusion if the healthcare provider is not aware of the specific overdose and the potential for assay interference.

Chronic Toxicity of Chloroquine and Hydroxychloroquine

The major chronic toxic effects of CQ and HCQ are retinopathy, neuromyopathy, and cardiomyopathy (16, 18, 26). As will be discussed, these generally relate to long-term use and substantial lifetime exposure to CQ and HCQ, as may occur with chronic therapy of autoimmune diseases. However, patients with certain pre-existing conditions may be more vulnerable to toxic effects at an earlier stage of therapy (7). An example would be patients who already have cardiomyopathy or who have a genetic syndrome associated

with cardiac arrhythmias such as long QT syndrome.

Two recent systematic reviews examined chronic toxic effects by CQ and HCQ (27, 28), with one being a meta-analysis of over 16 600 patients with rheumatic disease (28). Both noted that cardiac disorders were rare (assuming no acute overdoses) and typically seen in patients on long-term therapy with CQ or HCQ. Of the chronic toxicities, ocular effects have been the most extensively studied and are better understood than toxicities affecting the heart, nervous system, or muscle. Chronic toxicities are likely associated with bioaccumulation of the drugs and metabolites in tissues over months and years (27, 28). Table 1 summarizes symptoms of chronic toxicity and risk factors.

Chronic use of CQ and HCQ has been associated with ophthalmologic complications, specifically irreversible retinal injury due to the affinity of CQ and HCQ for melanin in the retinal pigment epithelium (26). Regular ophthalmologic examination is the standard of care for patients who are on these drugs long-term, as would be common for HCQ treatment of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus. Interestingly, ophthalmologic complications have not been reported from acute overdoses of CQ and HCQ, even near-fatal massive overdoses (14, 15, 20, 22); rather, the risk of eye injury correlates with the duration and overall total dosing of these medications.

As previously mentioned, daily doses of HCQ and CQ are approximately 400 mg in adults for the management of autoimmune diseases. This dosing will lead to exposure of approximately 1 kg of the drug during 7 years of therapy (7). Most investigations that have looked at patients on HCQ or CQ for <5 years have found the incidence of retinopathy to be lower than 2%. However, the incidence may be as high as 7.5% in those who have been on HCQ or CQ for >7 years (26). The risk of retinopathy is higher in Asians.

Measures to Promote Safe Use of Chloroquine and Hydroxychloroquine

There are multiple practical concerns with the use of HCQ and CQ in the treatment of COVID-19 (7). First, potentially millions of patients could receive treatment if HCQ is used as prophylaxis for COVID-19 or in non-hospitalized patients with less severe clinical course (29, 30). Even risks of low probability can lead to adverse effects if a wide group receives the medication. Second, the initial doses for treatment of COVID-19 are relatively high and may lead to toxicity in vulnerable patients. Third, the use of HCQ or CQ for treatment of COVID-19 may involve prescribers who are not very familiar with the adverse effect profile of these drugs. This would contrast with the use of HCQ by specialists treating autoimmune diseases (e.g., rheumatologists, dermatologists) or providers who have experience with CQ or HCQ for treatment of malaria and amebiasis. This risk illustrates the importance that the experimental use of CQ or HCQ for COVID-19 should occur in the context of clinical trials that can carefully monitor and document efficacy and safety. Last, the wide media attention to HCQ and CQ can lead to self-use of the drugs obtained from a variety of sources. People may ingest higher doses in a misguided attempt to achieve higher protection from infection or inadvertently due to confusion with dosing.

A primary goal in safe prescribing of CQ and HCQ is to consider clinical and epidemiological factors that may predispose patients to a higher risk of toxicity (7). The first important question is whether a patient has previously been treated with CQ or HCQ, and if so, the extent of that treatment, as lifetime exposure to the drug will influence the risk assessment for chronic toxicity. Pertinent previous medical history for the patient would be a history of ocular disorders, cardiac arrhythmias, cardiomyopathy, or neuromyopathy. Asian patients would have a higher baseline risk for the development of retinopathy (26). Patients should be periodically monitored, particularly for ophthalmologic and cardiac examination. There are proposed algorithms for assessment and monitoring of cardiovascular risk in patients who may receive HCQ or CQ. This routine monitoring may be challenging due to the shortage of healthcare providers and staff related to COVID-19. Patients should be instructed that new onset of symptoms such as vision difficulty, ocular pruritus (itching), and cardiovascular problems need to be reported quickly to the healthcare provider.

As described above, the metabolism of CQ and HCQ involves multiple CYP enzymes, leading to the possibility of drug-drug interactions (19). There is a particular risk with concomitant use of other medications that are CYP inhibitors, as these may lead to higher blood and tissue concentrations of CQ and HCQ. On the other hand, CQ and HCQ can reduce the activity of certain antibiotics and immunosuppressants (e.g., tacrolimus). CQ and HCQ therapy should be avoided in patients treated with mefloquine due to an increased risk of seizures. Drug-drug interactions become especially tricky in critically ill or transplant patients with COVID-19 who may have medication regimens that include antibiotics, immunosuppressants, and cardioactive drugs (19). The other risks for drug-drug interactions are those that increase the risk of specific organ toxicity. An example of this is the potential increased risk of cardiotoxicity posed by the combination of HCQ and azithromycin; in this case,

increased efficacy against COVID-19 needs to be weighed against the risk of adverse cardiac effects (31).

The medical complications associated with COVID-19 may themselves lead to a higher risk of therapy with HCQ and CQ (7). Up to one-third of hospitalized patients with COVID-19 have a concurrent cardiovascular injury resulting from direct viral injury, hypoxia, or thrombi. Patients infected with SARS-CoV-2 who have pre-existing cardiomyopathy or congestive heart failure are at an even higher risk of drug-related cardiac arrhythmias. Therefore, careful monitoring and follow-up of patients are important.

Discussion

HCQ and CQ have emerged as potential treatments for COVID-19. This is an example of repurposing drugs already in clinical use for other indications. The appeal of using these medications is their low cost and decades of clinical experience. The main downsides are the potential for adverse effects, including those arising from drug-drug interactions. The extensive coverage of these medications by the media has generated a lot of patient and healthcare interest. It is important to keep in mind their toxic effects and factors that may predispose to a higher risk of toxicity. Treatment of acute toxicity is supportive, and laboratory testing specifically for HCQ and CQ drug levels does not currently play a clinical role in acute toxicity management. Chronic toxicity is typically associated with years of medication exposure, although baseline risk factors such as cardiomyopathy increase risk.

Learning Objectives

After reading this article, the reader will be able to describe signs and symptoms associated with acute toxicity from chloroquine and hydroxychloroquine and factors that may increase the risk of toxicity. The reader will be able to identify adverse effects associated with long-term (chronic) use of chloroquine and hydroxychloroquine.

References

1. Greene CJ, Burleson SL, Crosby JC, Heimann MA, Pigott DC. Coronavirus disease 2019: international public health considerations. J Am Coll Emerg Physicians Open 2020, in press as doi: 10.1002/emp2.12040.
2. Zheng L, Zhang L, Huang J, Nandakumar KS, Liu S, Cheng K. Potential treatment methods targeting 2019-nCoV infection. Eur J Med Chem 2020;205:112687.
3. Group RC, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, Linsell L, Staplin N, Brightling C, Ustianowski A, et al. Dexamethasone in hospitalized patients with Covid-19–preliminary report. N Engl J Med 2020, in press as doi: 10.1056/NEJMoa2021436.
4. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, Li Y, Hu Z, Zhong W, Wang M. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov 2020;6:16.
5. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, Doudier B, Courjon J, Giordanengo V, Vieira VE, 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;56:105949.
6. Cavalcanti AB, Zampieri FG, Rosa RG, Azevedo LCP, Veiga VC, Avezum A, Damiani LP, Marcadenti A, Kawano-Dourado L, Lisboa T, et al. Hydroxychloroquine with or without Azithromycin in mild-to-moderate Covid-19. N Engl J Med 2020, in press as doi: 10.1056/NEJMoa2019014.
7. Pereira BB. Challenges and cares to promote rational use of chloroquine and hydroxychloroquine in the management of coronavirus disease 2019 (COVID-19) pandemic: a timely review. J Toxicol Environ Health B Crit Rev 2020;23:177–81.
8. Skipper CP, Pastick KA, Engen NW, Bangdiwala AS, Abassi M, Lofgren SM, Williams DA, Okafor EC, Pullen MF, Nicol MR, et al. Hydroxychloroquine in nonhospitalized adults with early COVID-19: a randomized trial. Ann Intern Med 2020, in press as doi: 10.7326/M20-4207.
9. U.S. Food & Drug Administration. Coronavirus (COVID-19) Update: Daily Roundup March 31, 2020. [https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-daily-roundup-march-31-2020]. Accessed August 29, 2020.
10. California State Board of Pharmacy, California Department of Consumer Affairs, and Medical Board of California. Statement Regarding Improper Prescribing of Medications Related to Treatment for Novel Coronavirus (COVID-19). [https://www.pharmacy.ca.gov/about/news_release/improper_prescribing.pdf]. Accessed August 29, 2020.
11. Man dies after ingesting chloroquine in an attempt to prevent coronavirus [https://www.ncbnews.com/health/healthnews/man-dies-after-ingesting-chloroquine-attempt-preventcoronavirus-n1167166]. Accessed August 29, 2020.
12. Ashley EA, Pyae Phyo A, Woodrow CJ. Malaria. Lancet 2018;391:1608–21.
13. World Health Organization Model List of Essential Medicines. 21st list. [https://www.who.int/medicines/publications/essentialmedicines/en/]. Accessed August 29, 2020.
14. Jaeger A, Sauder P, Kopferschmitt J, Flesch F. Clinical features and management of poisoning due to antimalarial drugs. Med Toxicol Adverse Drug Exp 1987;2:242–73.
15. Clemessy JL, Taboulet P, Hoffman JR, Hantson P, Barriot P, Bismuth C, Baud FJ. Treatment of acute chloroquine poisoning: a 5-year experience. Crit Care Med 1996;24:1189–95.
16. 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;23:231–69.
17. Hu C, Lu L, Wan JP, Wen C. The pharmacological mechanisms and therapeutic activities of hydroxychloroquine in rheumatic and related diseases. Curr Med Chem 2017;24:2241–49.
18. 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;38:653–71.
19. Zeitlinger M, Koch BCP, Bruggemann R, De Cock P, Felton T, Hites M, Le J, Luque S, MacGowan AP, Marriott DJE, et al. Pharmacokinetics/pharmacodynamics of antiviral agents used to treat SARS-CoV-2 and their potential

interaction with drugs and other supportive measures: a comprehensive review by the PK/PD of Anti-Infectives Study Group of the European Society of Antimicrobial Agents. Clin Pharmacokinet 2020, in press as doi: 10.1007/s40262-020-00924-9.
20. Jordan P, Brookes JG, Nikolic G, Le Couteur DG. Hydroxychloroquine overdose: toxicokinetics and management. J Toxicol Clin Toxicol 1999;37:861–64.
21. Clemessy JL, Favier C, Borron SW, Hantson PE, Vicaut E, Baud FJ. Hypokalaemia related to acute chloroquine ingestion. Lancet 1995;346:877–80.
22. Marquardt K, Albertson TE. Treatment of hydroxychloroquine overdose. Am J Emerg Med 2001;19:420–4.
23. Graham JD. An overdose of "plaquenil". Br Med J 1960;1:1256.
24. Radke JB, Kingery JM, Maakestad J, Krasowski MD. Diagnostic pitfalls and laboratory test interference after hydroxychloroquine intoxication: A case report. Toxicol Rep 2019;6:1040–6.
25. da Silva AS, Falkenberg M. Analytical interference of quinolone antibiotics and quinine derived drugs on urinary protein determined by reagent strips and the pyrogallol red-molybdate protein assay. Clin Biochem 2011;44:1000–4.
26. Melles RB, Marmor MF. The risk of toxic retinopathy in patients on long-term hydroxychloroquine therapy. JAMA Ophthalmol 2014;132:1453–60.
27. Chatre C, Roubille F, Vernhet H, Jorgensen C, Pers YM. Cardiac complications attributed to chloroquine and hydroxychloroquine: a systematic review of the iterature. Drug Saf 2018;41:919–31.
28. Liu D, Li X, Zhang Y, Kwong JS, Li L, Zhang Y, Xu C, Li Q, Sun X, Tian H, et al. Chloroquine and hydroxychloroquine are associated with reduced cardiovascular risk: a systematic review and meta-analysis. Drug Des Devel Ther 2018;12:1685–95.
29. Medina MT, Moncada SS. Hydroxychloroquine/chloroquine as a treatment choice or prophylaxis for Covid-19 at the primary care level in developing countries: a primum non nocere dilemma. J Neurol Sci 2020;415:116972.
30. Ullah W, H MA, Roomi S, Sattar Y, Almas T, Narayana Gowda S, Saeed R, Mukhtar M, Ahmad A, Oliver T, et al. Safety and efficacy of hydroxychloroquine in COVID-19: a systematic review and meta-analysis. J Clin Med Res 2020;12:483–91.
31. Aggarwal G, Henry BM, Aggarwal S, Bangalore S. Cardiovascular safety of potential drugs for the treatment of coronavirus disease 2019. Am J Cardiol 2020;128:147–50.

Matthew D. Krasowski, MD, PhD, is a Clinical Professor and Vice-Chair of Clinical Pathology and Laboratory Services in the Department of Pathology at the University of Iowa Hospitals and Clinics. E-mail: mkrasows@healthcare.uiowa.edu.

The author has nothing to disclose.

FDA Alerts Consumers to Toxic Hand Sanitizers

By Bridgit O. Crews, PhD, DABCC

To help prevent the spread of COVID-19, the Centers for Disease Control and Prevention (CDC) recommends using hand sanitizers that contain at least 60% ethanol, or 70% isopropanol, when soap and water are not available (1). Both ethanol and isopropanol (2-propanol) have been shown to be effective against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as they inactivate the enveloped virus (2). Benzalkonium chloride, which is also present in some hand sanitizers, may be less effective. Early in the SARS-CoV-2 pandemic, high demand for ethanol-containing hand sanitizer led to shortages and in response, new formulations and manufacturers appeared on the market. Hand sanitizers are considered over-the-counter drugs, but given the unique situation, the FDA issued emergency guidance on their preparation, as well as guidance on the manufacture of the alcohols used in them. Some distilleries shifted manufacturing to fill the need, and imports also increased.

Between May and June 2020, the CDC reported 15 cases of methanol poisonings due to the ingestion of hand sanitizers in Arizona and New Mexico. Four of the individuals died and three were discharged with visual impairments (3). On June 19, 2020, the FDA issued an alert to warn consumers to the presence of methanol in some hand sanitizers. Tested sanitizers contained as much as 81% methanol (v/v) (4). Since then the FDA has issued additional alerts and is constantly expanding the list of potentially contaminated hand sanitizers. Most recently, the FDA reported findings of 1-propanol in other hand sanitizers on August 12, 2020, as well.

The primary risk of toxicity from contaminated hand sanitizers is by accidental or intentional ingestions. Younger children are especially at risk of accidental ingestion, but poisonings due to intentional ingestion also occur when adolescents or adults ingest hand sanitizer as a substitute for alcohol (5). In March 2020, calls to poison control centers related to hand sanitizers increased by 79% compared to 2019, with most calls concerning unintentional ingestion by children under age five (4). To deter both accidental ingestion as well as substitutional ingestion, hand sanitizers should contain denaturants to make the scent and flavor less appealing. Colorful packaging is also discouraged.

Depending on the dose, methanol exposure may cause nausea, headache, blurred vision or permanent blindness, seizure, coma, and death. The toxicity of methanol occurs primarily through the accumulation of toxic metabolites rather than methanol itself (6). Methanol toxicity may initially present as drowsiness with some nausea, with severe toxicological symptoms such as metabolic acidosis and visual impairment being delayed by up to 30 h, after the accumulation of toxic metabolites. Mixed alcohol ingestion may also delay more severe symptoms, as ethanol is a competitive substrate for alcohol dehydrogenase, and its presence inhibits

the metabolism of methanol. An ingestion as small as 10 mL of methanol may result in permanent blindness and the ingestion of 100 to 200 mL is expected to be fatal in most adults (7). Early identification of exposure and treatment with fomepizole (4-methylpyrazole) to prevent metabolism to formic acid through competitive inhibition of alcohol dehydrogenase is crucial for the prevention of irreversible sequela. Depending on the dose and clinical symptoms hemodialysis may also be required.

Compared to ethanol, 1-propanol is rapidly absorbed and is a more potent central nervous system depressant. Signs and symptoms of toxicity appear rapidly following the ingestion of 1-propanol and include confusion, decreased consciousness, and slowed pulse and breathing (8). 1-Propanol has a linear structure and is metabolized by alcohol dehydrogenase to propionic acid. Toxicity can, therefore, result in metabolic acidosis with an elevated anion gap. Propionic acid may be further metabolized to lactate. Reported fatalities from 1-propanol are rare, and a lethal dose is not known but is likely lower than the estimated lethal dose for 2-propanol (isopropanol), which is estimated at 240 mL for an adult. Unlike 1-propanol, isopropanol does not result in acidosis, but may present with ketosis and ketonuria resulting from its metabolism to acetone (4).

Minimal amounts of nontoxic concentrations of alcohol from hand rubs may be absorbed dermally, or by inhalation of vapors after a single use (9). There is less data on toxicity resulting from frequent repeated use, but there are reports of systemic neurotoxicity due to repeated dermal exposures (10). Children may also be at higher risk due to higher skin surface area to body mass ratio.

No consumer hand sanitizers are approved by the FDA, and the labeling of a hand sanitizer with any fraudulent claim such as "FDA approved," "prevents COVID-19," or "works for up to 24 hours" is considered a sign of a potentially hazardous product. Although recipes for homemade hand sanitizers can be found online, this is not recommended, as homemade hand sanitizers may be ineffective and have reportedly resulted in skin burns and irritation. The FDA also recently updated guidance to clarify the requirement that companies that produce hand sanitizers test each lot of ethanol or isopropanol active ingredient that is used if the active ingredient is obtained from an outside source.

At the time of this writing, there were over 165 products listed on the FDA website, from over 59 different listed manufacturers. Sources of manufactures included Mexico, China, and five US states. Some of these products have been sold in major retail stores. A list of hand sanitizers with known or suspected contamination can be accessed on the FDA website (11).

Learning Objectives

After reading this article, the reader will be able to describe the signs and symptoms of a toxic methanol or 1-propanol ingestion. The reader will be able to explain the risk of toxic exposure resulting from hand sanitizers contaminated with methanol or 1-propanol.

References

1. Centers for Disease Control and Prevention. (2020). Hand Hygiene Recommendations [Online]. Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/hand-hygiene.html#references (Accessed August 20, 2020).
2. Kratzel A, Todt D, V'kovski P, Steiner S, Gultom M, Thao TTN, et al. Inactivation of severe acute respiratory syndrome coronavirus 2 by WHO-recommended hand rub formulations and alcohols. Emerg Infect Dis 2020;26:1592–95.
3. Yip L, Bixler D, Brooks DE, Clarke KR, Datta SD, Dudley S Jr, et al. Serious adverse health events, including death, associated with ingesting alcohol-based hand sanitizers containing methanol—Arizona and New Mexico, May–June 2020. MMWR Morb Mortal Wkly Rep 2020;69:1070–73.
4. US Food and Drug Administration. (2020). FDA continues to ensure availability of alcohol-based hand sanitizer during the COVID-19 pandemic, addresses safety concerns [Online]. Available at: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-continues-ensure-availability-alcohol-based-hand-sanitizer-during (Accessed August 20, 2020).
5. Darracq MA, Ghafouri N, Pesce A, Cantrell FL. Hand sanitizer intoxication following a crude extraction method. Am J Drug Alcohol Abuse 2013;39:217–8.
6. Greene HR, Krasowski MD. (March 2020). Ingestion of toxic alcohols and glycols: an ongoing diagnostic and analytical challenge. Clinical and Forensic Toxicology News (Quarterly, AACC/CAP), 1-6. Available from: https://www.aacc.org/publications/clinical-and-forensic-toxciology-news.
7. Basaelt RC, Cravey RH. (1995) Disposition of toxic drugs and chemicals in man 4th ed. Chemical Toxicology Insititute.
8. Vujasinovic M, Kocar M, Kramer K, Bunc M, Brvar M. Poisoning with 1-propanol and 2-propanol. Hum Exp Toxicol 2007;26:975–8.
9. Below H, Partecke I, Huebner NO, Bieber N, Nicolai T, Usche A, et al. Dermal and pulmonary absorption of propan-1-ol and propan-2-ol from hand rubs. Am J Infect Control 2012;40:250–7.
10. Chan APL, Chan TYK. Methanol as an unlisted ingredient in supposedly alcohol-based hand rub can pose serious health risk. Int J Environ Res Public Health 2018;15:1440–5.
11. US Food and Drug Administration. (2020). FDA updates on hand sanitizers consumers should not use [Online]. Available at: www.fda.gov/unsafehandsanitizers (Accessed August 20, 2020).

Bridgit O. Crews, PhD, DABCC, TC, is an associate clinical professor of pathology and laboratory medicine and director of clinical chemistry and toxicology at the University of California Irvine Medical Center in Orange as well as Chair of

the Clinical & Forensic Toxicology News *board of editors. E-mail: crewsb@uci.edu.*

The author has nothing to disclose.

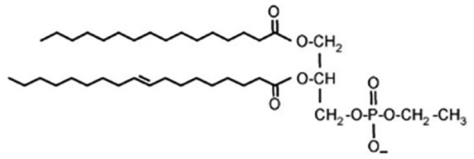
Phosphatidylethanol: A Long-Term Biomarker for Ethanol Exposure

By Kamisha L. Johnson-Davis, PhD

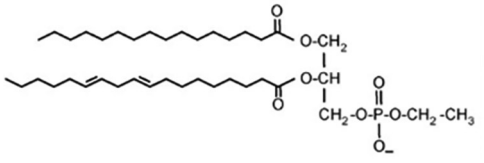
Alcohol use disorders are a major health concern across the globe. Data from the 2018 National Survey on Drug Use and Health estimated that 139.8 million Americans 12 years or older were current alcohol users (about 51% of the population), which was defined as any use of alcohol in the past 30 days (1). The survey also identified 67.1 million (or 48% of alcohol users) as binge drinkers, which was defined as drinking 5 or more drinks for males and 4 or more drinks for females on the same day, within the past 30 days (1). Heavy alcohol users were defined as individuals that binge drank for 5 or more days, in the past 30 days. It was estimated that 16.6 million Americans were classified in this category (1). Testing for ethanol exposure is important for patient care to identify individuals with alcohol use disorders.

Direct biomarkers for alcohol exposure, such as ethyl glucuronide and ethyl sulfate have high specificity, but the window of detection is <1 week in urine, and ethanol in blood and urine has a window of detection of <24 h (2). Indirect biomarkers for alcohol exposures, such as liver enzymes, gamma glutamyltransferase, aspartate aminotransferase, and alanine aminotransferase can increase as a result of heavy alcohol use; however, these liver enzymes can also increase due to liver injury or disease unrelated to alcohol use disorders; therefore, these enzymes are not specific for alcohol consumption (2). Carbohydrate-deficient transferrin (CDT) is a specific and indirect biomarker for heavy drinking, defined as daily consumption of 50 to 80 g/day of alcohol (approximately 4 standard drinks per day) over several weeks. Heavy alcohol consumption will decrease the glycosylation of transferrin to produce, disialotransferrin, monosialotranferrin, and asialotransferrin (free of sialic-acid) forms (3). CDT will reduce to normal concentrations after about 14 days of alcohol cessation. However, CDT is not a sensitive marker to detect light to moderate alcohol users and false-positive CDT results can occur in physiological conditions such as liver disease, congenital disorders of glycosylation, genetic variants of transferrin (B, C, and D), high total transferrin concentrations, pregnancy, iron-deficiency anemia, and pancreas and liver transplantation (2-4). False-negative CDT results can occur due to low transferrin concentrations and transferrin B variants (3).

Consequently, there is a clinical need for a direct alcohol biomarker that is highly specific and with a longer window of detection for acute and chronic exposure. Phosphatidylethanol (PEth) is a direct ethanol marker that detects acute and chronic heavy drinking in blood with high specificity. Phosphatidylethanol is a phospholipid formed in the red blood cell (RBC) membrane in the presence of ethanol and the enzyme phospholipase D (PLD) (5). PLD has approximately 1000-fold greater affinity for ethanol than water; therefore, in the presence of ethanol, PLD catalyzes a transphosphatidylation reaction of phosphatidylcholine to form PEth homologs. In the absence of ethanol in the blood, PLD hydrolyzes phosphatidylcholine to produce phosphatidic acid and choline. PEth is formed in tissues such as the brain, liver, platelets, lymphocytes, and RBCs. RBCs do not have the enzyme phosphatidylcholine phospholipase C, which degrades PEth, therefore, the half-life of PEth in the blood is prolonged in comparison to other tissues. There are 48 homologs of PEth that have been identified and are distinguished by the number of carbons and double bonds in the fatty acid chains (6). PEth homologs have a common nonpolar phosphoethanol head group with 2 fatty acid moieties. 1-Palmitoyl-2-oleoyl-phosphatidylethanol (PEth 16:0/18:1), and 1-palmitoyl-2-linoleoyl-phosphatidylethanol (PEth 16:0/18:2) are the predominant PEth homologs in whole blood and makeup about 60% of the total PEth species (Figure 1). PEth is detectable after drinking 1 to 2 alcoholic beverages and experiments have shown that PEth was detected in blood after 30 min and peaked after 90 to 120 min (7). The half-life is estimated to be 3 to 5 days; however, the half-life is variable among patients due to inter-individual variation in the formation and elimination of the PEth homologs



PEth-16:0/18:1*



PEth-16:0/18:2

Figure 1. Phosphatidylethanol homologue structures.

Table 1. Phosphatidylethanol result interpretation

PEth (16:0/18:1) whole blood concentration	Result interpretation (14)
<20 ng/mL	Abstinence or light drinking (<2 drinks per day for several days a week)
20–200 ng/mL	Moderate drinking (up to 4 drinks per day for several days a week)
>200 ng/mL	Heavy drinking (at least 4 drinks per day several days a week)

(8). The window of detection can span up to approximately 28 days or more for individuals who are heavy, chronic alcohol drinkers (5, 9, 10). The formation of PEth is proportional to the concentration of ethanol at the site of PLD, PLD activity (11), and the rate of intestinal absorption of ethanol (7).

Studies have shown that PEth has improved sensitivity and specificity to detected alcohol exposure in comparison to CDT, ethyl glucuromide/ethyl sulfate, and liver enzyme biomarkers (12). Clinical testing for PEth may be used to support patient care to prequalify individuals for organ transplantation, to assess drinking during pregnancy or detect neonatal drug exposure, and to monitor compliance for alcohol abstinence programs. Commercial kits for the detection of PEth homologs are currently not available; therefore analysis is primarily performed by laboratory-developed mass spectrometry methods. The concentration of PEth in whole blood may be utilized to distinguish chronic or heavy drinkers from social/light drinkers. Cutoffs to interpret light, moderate, and heavy drinkers are not well established; however, the consensus cutoffs used are based on clinical studies that demonstrate patients identified as heavy drinkers tend to have whole blood PEth concentrations >200 ng/mL (13) (Table 1). Therefore, more clinical studies are needed to establish interpretive cutoffs for abstinence, moderate drinking, and to assess PEth concentrations in patients with liver disease.

Learning Objectives

After reading this article, the reader will be able to describe biomarkers commonly used to detect alcohol exposure and the benefit of a long-term marker to monitor alcohol abstinence.

References

1. NIDA, National Survey on Drug Use and Health (NSDUH). 2018. https://www.samhsa.gov/data/sites/default/files/cbhsq-reports/NSDUHffrBriefingSlides2018_w-final-cover.pdf (Accessed August 2020).
2. Nanau RM, Neuman MG. Biomolecules and biomarkers used in diagnosis of alcohol drinking and in monitoring therapeutic interventions. Biomolecules 2015;5:1339–85.
3. Helander A, Eriksson G, Stibler H, Jeppsson JO. Interference of transferrin isoform types with carbohydrate-deficient transferrin quantification in the identification of alcohol abuse. Clin Chem 2001;47:1225–33.
4. Kenan N, Larsson A, Axelsson O, Helander A. Changes in transferrin glycosylation during pregnancy may lead to false-positive carbohydrate-deficient transferrin (CDT) results in testing for riskful alcohol consumption. Clin Chim Acta 2011;412:129–33.
5. Viel G, Boscolo-Berto R, Cecchetto G, Fais P, Nalesso A, Ferrara SD. Phosphatidylethanol in blood as a marker of chronic alcohol use: a systematic review and meta-analysis. Int J Mol Sci 2012;13:14788–812.
6. Gnann H, Engelmann C, Skopp G, Winkler M, Auwärter V, Dresen S, et al. Identification of 48 homologues of phosphatidylethanol in blood by LC-ESI-MS/MS. Anal Bioanal Chem 2010;396:2415–23.
7. Javors MA, Hill-Kapturczak N, Roache JD, Karns-Wright TE, Dougherty DM. Characterization of the pharmacokinetics of phosphatidylethanol 16:0/18:1 and 16:0/18:2 in human whole blood after alcohol consumption in a clinical laboratory study. Alcohol Clin Exp Res 2016;40:1228–34.
8. Hill-Kapturczak N, Dougherty DM, Roache JD, Karns-Wright TE, Javors MA. Differences in the synthesis and elimination of phosphatidylethanol 16:0/18:1 and 16:0/18:2 after acute doses of alcohol. Alcohol Clin Exp Res 2018;42:851–60.
9. Schröck A, Thierauf-Emberger A, Schürch S, Weinmann W. Phosphatidylethanol (PEth) detected in blood for 3 to 12 days after single consumption of alcohol-a drinking study with 16 volunteers. Int J Legal Med 2017;131:153–60.
10. Fleming MF, Smith MJ, Oslakovic E, Lucey MR, Vue JX, Al-Saden P, Levitsky J. Phosphatidylethanol detects moderate-to-heavy alcohol use in liver transplant recipients. Alcohol Clin Exp Res 2017;41:857–62.
11. Aradóttir S, Moller K, Alling C. Phosphatidylethanol formation and degradation in human and rat blood. Alcohol Alcohol 2004;39:8–13.
12. Walther L, de Bejczy A, Löf E, Hansson T, Andersson A, Guterstam J, et al. Phosphatidylethanol is superior to carbohydrate-deficient transferrin and γ-glutamyltransferase as an alcohol marker and is a reliable estimate of alcohol consumption level. Alcohol Clin Exp Res 2015;39:2200–8.
13. Hahn JA, Anton RF, Javors MA. The formation, elimination, interpretation, and future research needs of phosphatidylethanol for research studies and clinical practice. Alcohol Clin Exp Res 2016;40:2292–95.
14. Ulwelling W, Smith K. The PEth blood test in the security environment: what it is; why it is important; and interpretative guidelines. J Forensic Sci 2018;63:1634–40.

Kamisha L. Johnson-Davis, PhD, is an Associate Professor (Clinical) at the University of Utah School of Medicine and Medical Director for Clinical Toxicology at ARUP Laboratories. She is certified by the American Board of Clinical

Chemistry in Clinical Chemistry and Toxicological Chemistry. Dr. Johnson-Davis is the Director for the Clinical Chemistry Fellowship Program at the University of Utah, and she currently serves as a ComACC Commissioner. E-mail: kamisha.johnson-davis@aruplab.com.

The author has nothing to disclose.

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