Journal of Medical Toxicology (2020) 16:284–294
https://doi.org/10.1007/s13181-020-00777-5

REVIEW

COVID-19: Therapeutics and Their Toxicities

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Received: 24 March 2020 / Revised: 9 April 2020 / Accepted: 9 April 2020 / Published online: 30 April 2020

Abstract

SARS-CoV-2 is a novel coronavirus that emerged in 2019 and is causing the COVID-19 pandemic. There is no current standard of care. Clinicians need to be mindful of the toxicity of a wide variety of possibly unfamiliar substances being tested or repurposed to treat COVID-19. The United States Food and Drug Administration (FDA) has provided emergency authorization for the use of chloroquine and hydroxychloroquine. These two medications may precipitate ventricular dysrhythmias, necessitating cardiac and electrolyte monitoring, and in severe cases, treatment with epinephrine and high-doses of diazepam. Recombinant protein therapeutics may cause serum sickness or immune complex deposition. Nucleic acid vaccines may introduce mutations into the human genome. ACE inhibitors and ibuprofen have been suggested to exacerbate the pathogenesis of COVID-19. Here, we review the use, mechanism of action, and toxicity of proposed COVID-19 therapeutics.

Keywords COVID-19 · SARS-CoV-2 · Toxicity · Pandemic · Therapeutic

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that causes *co*rona*vi*rus *d*isease 2019 (COVID-19), an infection characterized by flu-like symptoms, progressing in some cases to acute respiratory distress syndrome (ARDS) [1, 2] or myocarditis [3–6]. COVID-19 was classified as a pandemic by the World Health Organization (WHO) on March 11, 2020. The disease has reached nearly every country. As of April 16, 2020 there were 2,090,110 confirmed cases worldwide, with 139,469 deaths [7]. The death and disability from COVID-19, combined with the lack of approved treatments, have created an unmet need for efficacious therapies.

As researchers and pharmaceutical companies race to develop vaccines and antivirals, some have turned to remedies with little to no supporting evidence. At least 300 Iranians have died and 1,000 have sustained blindness and brain damage from methanol poisoning, in the mistaken belief that alcohol could prevent coronavirus infection [8]. Social media have propagated the misinformation that snorting cocaine or bleach will prevent infection [9–11]. The desperation of these acts speaks to an urgent, unmet need for effective therapeutics against SARS-CoV-19.

We intend for this review to familiarize readers with the toxicities of potential therapeutics for COVID-19 and possible treatments for those toxicities. This review draws on multiple sources, combining news articles, social media posts, and peer-reviewed research. We identified the substances included in this review based on discussion in the media and our experience as practicing physicians, scientists, and pharmacists. We rely on industry or news reports if no other source of information was available. Our discussion here presents our knowledge of novel therapeutics as publicly known in early April 2020.

Supervising Editor: Mark B. Mycyk, MD

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This article will discuss some therapeutic options against SAR-CoV-2, using the life-cycle of SARS-CoV-2 to understand the mechanism and toxicity of each agent. We will discuss (1) viral cell entry inhibitory (e.g., chloroquine, hydroxychloroquine, APN01), (2) viral replication inhibitors (e.g., protease inhibitors, nucleotide analogs), (3) nucleic acid vaccines, and (4) miscellaneous medications (azithromycin, ACE inhibitors, and ibuprofen).

Biology of Coronavirus

SARS-CoV-2 is an enveloped non-segmented positive-sense RNA virus that belongs to the *Coronaviridae* family. To date, seven coronaviruses are known to infect humans (229E, NL63, OC43, HKU1, MERS-CoV, SARS-CoV-1) with three (SARS-CoV-1, MERS-CoV, and SARS-CoV-2) causing severe disease in humans [3]. The genetic sequence of SARS-CoV-2 closely resembles that of SARS-CoV-1, a beta-coronavirus that caused the severe acute respiratory syndrome (SARS) epidemic in 2003. Much of our current understanding of SARS-CoV-2 is based off in vitro and in vivo experiments conducted to investigate the pathogenesis of SARS-CoV-1.

Coronavirus spreads between humans through respiratory droplets. SARS-CoV-1 and SARS-CoV-2 gain entry when the spike protein on the surface of the viral capsid binds to angiotensin-converting enzyme II (ACE2) on type II alveolar cells, leading to fusion of the viral and host cell membranes, and injection of the viral RNA into the host cytoplasm [12]. Once inside the cell, the virus replicates its genetic material and then releases newly created virus particles (virions). Each of these steps is an opportunity for treatments to disrupt the normal coronavirus life cycle.

Therapeutics

Researchers are currently investigating treatments for COVID-19, by developing vaccines and novel drugs as well as testing existing medications. A vaccine against SARS-CoV-2 may be clinically available in 12–18 months [13]. Medications approved by the Food and Drug Administration (FDA) for other purposes,s such as chloroquine, are being repositioned to treat COVID-19. Therapeutic repurposing (i.e., repositioning) is an expedited drug-development strategy to reuse currently FDA-approved therapeutics for new medical indications. On March 28, the FDA granted emergency authorization for the use of chloroquine phosphate and hydroxychloroquine sulfate to treat COVID-19 [14]. The European Medicines Agency recommended restricting their use pending the outcome of clinical trials [15]. The American Academy of Clinical Toxicology (AACT), the American Association of Poison Control Centers (AAPCC), and the American College of Medical Toxicology (ACMT) recommended in a joint press release that use of chloroquine and hydroxychloroquine should occur only under the direction of a medical provider, for an FDA-approved indication, or as part of a trial for the treatment of COVID-19 or as part of an approved hospital protocol [16].

Our discussion does not cover all drugs, and is not intended to be exhaustive. In the absence of direct evidence, we expect drugs of a similar class to have similar toxicity.

Viral Entry Inhibitors

Chloroquine and Hydroxychloroquine

Chloroquine is a 4-aminoquinoline primarily used to treat malaria, an infectious disease caused by several *Plasmodia* species. Chloroquine concentrates in acidic environments, such as the digestive vacuole of *Plasmodia* spp. or the Golgi apparatus of human cells. Chloroquine prevents *Plasmodia* from crystallizing heme to hemozoin [17], leading to a buildup of heme that becomes toxic to the parasite. The Golgi apparatus is a collection of vesicles where post-translational modifications such as glycosylation occur.

Chloroquine freely diffuses into vacuoles. The acidic environment of the vacuole favors the protonated (charged) form, which cannot freely diffuse away (*ion trapping*), leading to a buildup of chloroquine in the vacuoles. Chloroquine alters the glycosylation of ACE2, which decreases the affinity of ACE2 for the coronavirus spike protein, reducing SARS-CoV-2 entry in vitro [18]. Additionally, chloroquine and hydroxychloroquine inhibit the Toll-like receptor (TLR) pathway; the TLR pathway is involved in pro-inflammatory cytokine signaling [19].

Chloroquine was found to inhibit SARS-CoV-1 infection and he sequence and structure homologies between SARS-CoV-1 and SARS-CoV-2 suggest that chloroquine could reduce SARS-CoV-2 infectivity [18]. Indeed, one in vitro study demonstrated that chloroquine inhibits SARS-CoV-2 entry [21]. Guangdong province recommended 500 mg chloroquine twice a day for 10 days for any person diagnosed with COVID-19 without contraindications to chloroquine [20]. One abstract/commentary suggests efficacy, but cites an audio transcript of a press conference in Guangdong, China, the supporting data for which is not available [22].

Doses greater than 5 grams of chloroquine are associated with mortality due to ventricular dysrhythmias and hypokalemia [23]. Cardiovascular collapse and profound hypotension can occur within 1–3 hours of overdose; sodium channel blockade results in QRS widening on ECG [24-26]. Neurologic effects include seizures and CNS depression [27]. Oxidative stress can lead to hemolysis, particularly in patients with G6PD deficiency. Potassium channel blockade can result in prolonged QTc interval and torsades de pointes, and

clinicians should avoid using QT prolonging agents if chloroquine toxicity is present.

In this current pandemic, a man in Arizona died after ingesting chloroquine phosphate, a form of chloroquine used for treating aquariums, using it to self-medicate [24], and cases of chloroquine poisoning have been reported in Nigeria [25]. It is important to store chloroquine safely to avoid secondary harm. As little as 10 mg/kg of chloroquine in children, which could be 1–2 pills, requires medical evaluation; 27 mg/kg was the lowest fatal dose in toddlers [28].

Hydroxychloroquine is a derivative of chloroquine. It is considered less toxic. The difference in mechanisms of action between chloroquine and hydroxychloroquine is not fully understood. Chloroquine and hydroxychloroquine are dealkylated into desethylchloroquine by CYP3A4/5 and 2C8 [29]. Hydroxychloroquine is also dealkylated into desethylhydroxychloroquine by 2D6 [19]. Chloroquine and hydroxychloroquine inhibit 2D6, which can increase serum concentrations of metoprolol, propranolol, opiates, antidysrhythmics, antidepressants, and antipsychotics [30].

The minimum fatal dose of hydroxychloroquine is not well defined. The minimum dose reported to elicit severe symptoms (hypotension, hypokalemia, and ventricular dysrhythmias) is 4 g [31]. One case report from 1965 (for which the full text is not available) describes a 16 year old who died after reportedly ingesting 12 g of hydroxychloroquine [32]. An 18-year-old girl who ingested 20 g of hydroxychloroquine developed hypokalemia, hypotension, and ventricular tachycardia but survived with intubation, epinephrine infusion, high-dose diazepam, and potassium repletion [33] (see "Treatment of Chloroquine and Hydroxychloroquine Toxicity" section). Three other case reports describe a total of five severely symptomatic patients who survived overdoses of 20–36 g of hydroxychloroquine with aggressive interventions, including epinephrine and high-dose diazepam [33–36].

Data regarding the efficacy of hydroxychloroquine in treating COVID-19 are limited. One clinical trial, that has not been peer reviewed, suggests that in patients with a clinical diagnosis of COVID-19, 25 out of 31 who received hydroxychloroquine clinically improved after 5 days as compared with 17 out of 31 in the control group [37]. In that study, one patient who received hydroxychloroquine developed a rash without mucosal involvement and another developed a headache. Both resolved without intervention.

Treatment of Chloroquine and Hydroxychloroquine Toxicity

In 1988, Riou et al. published a prospective study of 11 patients who were anticipated to have a likely lethal ingestion of chloroquine (greater than 5 g of chloroquine) and compared them to 11 historical controls. These individuals were treated with epinephrine infusion (initial 0.25 μg/kg/min, titrated to SBP > 100 mmHg), 2 mg/kg of diazepam over 30 min, intubation, and nasogastric aspiration. Diazepam was continued at 1–2 mg/kg per day over 2–4 days. Ten of these 11 patients survived to hospital discharge, compared with 1 out of 11 patients in the control group [23]. Diazepam, perhaps not intuitively, was incorporated in this combination therapy due to animal [38] and human evidence that it may be beneficial in combined overdoses. This study has largely shaped the recommended treatment of epinephrine and high dose diazepam, though not without some controversy regarding its generalizability.

Hypokalemia is a common finding in chloroquine and hydroxychloroquine overdose and is likely due to intracellular shift of potassium. In a retrospective study of 191 patients with chloroquine toxicity, a serum potassium lower than 3.0 mmol/L was correlated with mortality, and the serum potassium decreased as serum chloroquine levels increased [39]. The linear relationship between serum potassium and chloroquine concentrations suggests that hypokalemia reflects an effect of chloroquine itself, although the severely ill patients also received epinephrine infusions (beta-adrenergic agonists can cause intracellular potassium shifts). Total body potassium stores are not expected to be depleted in the setting of chloroquine toxicity, raising concern for rebound hyperkalemia as toxicity resolves. As such, some clinicians recommend less aggressive potassium repletion, supplementing normal daily potassium with 80 mEq per 24 h [39], though if severe hypokalemia is present, it would be reasonable to replete, while observing for rebound hyperkalemia. The treatment for acute toxicity from hydroxychloroquine is thought to be the same as for acute toxicity from chloroquine.

QRS prolongation from xenobiotic-induced sodium channel blockade is often treated with sodium bicarbonate boluses and infusions. Administration of sodium bicarbonate may treat the cardiac sodium channel blockade but worsen hypokalemia, so patient-specific characteristics must be considered when weighing the use of this therapy.

Lipid emulsion therapy did not lead to return of spontaneous circulation when given during cardiac arrest for either a hydroxychloroquine ingestion or a combined ingestion of hydroxychloroquine and chloroquine [40]. Two case reports suggest that lipid emulsion can reverse hypotension averting cardiac arrest [41, 42]. In the second case report, one patient received lipid emulsion therapy and did not develop significant cardiotoxicity beyond a prolonged QTc interval, although this patient ingested a smaller dose of hydroxychloroquine (5 g) [42]. Extracorporeal circulatory support could be considered for severe overdoses refractory to other treatments [43].

Chloroquine and hydroxychloroquine can both lead to retinopathy. It is rare for this toxicity to develop before 5 years of therapy [44], and is not expected with short-term use. Of 3995

patients with rheumatoid arthritis who ever used hydroxychloroquine, 6.5% discontinued it because of "eye complaints," although probable or definite retinal toxicity was documented in less than 1%; the risk of retinal toxicity increased by fivefold once lifetime exposure reached 1 kg [44]. Long-term use of hydroxychloroquine has also been associated with cardiomyopathy [45, 46].

Clinicians should also be mindful of secondary effects of an increase in demand for chloroquine and hydroxychloroquine to treat COVID-19. Hydroxychloroquine is used to treat systemic lupus erythematosus (SLE). Some patients with SLE have been unable to fill their prescriptions due to shortages [47]. Some state pharmaceutical boards have noted surges in prescriptions for hydroxychloroquine, chloroquine, and azithromycin written by physicians for either themselves or their family members, a practice which the American Medical Association promptly denounced [48].

APN01

APN01 (Apeiron Biologics) is a recombinant human ACE2 protein first developed to treat SARS [49]. It may treat COVID-19 by preventing SARS-CoV-2 entry and reducing acute lung injury. It does so by acting as a soluble molecular decoy for SARS-CoV-2, preventing the virus from binding to cellular ACE2 in a dose-dependent fashion [50]. ACE2, in vitro, is the essential receptor for the SARS-CoV-2 entry [51] and also serves to maintain normal lung physiology. When the virion binds to cell surface ACE2 this lung protective signalingis lost. By preventing ACE2-mediated SARS-CoV-2 interaction, and therefore restoring physiologic ACE2 signaling, APN01 may reduce acute lung injury [52]. A randomized unblinded clinical trial is underway in China to investigate the efficacy of APN01 to reduce viral load and the duration of COVID-19 symptoms [53].

A phase I trial of APN01 from 2013 by Haschke and colleagues was well-tolerated with no cardiovascular side effects in 22 healthy subjects. Toxicity from recombinant proteins, in general, arises from inadvertent activation of the immune system (unintentional immunogenicity) and binding of the recombinant proteins to other cell surface receptors triggering cellular signal transduction cascades that were not consciously targeted (off-target binding). Unintentional immunogenicity can lead to the formation of anti-drug antibodies that can deposit in tissues as immune complexes. For example, immune complexes can deposit in the kidney impairing the kidney's ability to filter out toxins and recover electrolytes. If the immune system treats the recombinant protein as an antigen, treatment may precipitate serum sickness. Off-target binding of APN01 could modulate renin-angiotensin signaling, altering blood pressure and organ perfusion. One abstract provides evidence that up to 1.2 mg/kg per day caused no immunogenicity or cardiovascular toxicity in three healthy human volunteers [54].

Leronlimab (PRO 140)

Leronlimab (CytoDyn) is an investigational humanized IgG4 monoclonal antibody against CCR5 receptors found on T lymphocytes. Chemokine receptor 5 (CCR5) was first characterized for its role as a co-receptor in human immunodeficiency virus (HIV) viral entry into white blood cells [55]. We now recognize that other pathogens, such as Dengue [56] or *Staphylococcus aureus* [57] also use the CCR5 signaling pathway for entry or as a virulence factor. Leronlimab is being repurposed and investigated as a treatment option for patients with COVID-19 who experience respiratory complications as a result of COVID-19. A single-arm, open-label, multi-center clinical study is set to take place to investigate the clinical improvement in total symptom score (i.e., fever, myalgia, dyspnea, and cough). Currently, leronlimab has a "fast-track" designation from the FDA for HIV and metastatic triple-negative breast cancer [58]. There are no serious side effects or adverse events reported so far. Leronlimab has successfully completed nine phase 1, 2, and 3 clinical trials in about 800 patients for other indications and achieved primary efficacy endpoints [59].

Reasoning by analogy with approved therapeutics in oncology, the acute toxicity from humanized antibodies can include (1) immunosuppression, predisposing to opportunistic infections or viral-induced neoplasias; (2) immunostimulation, including the dramatic "cytokine storm" of fevers, chills, myalgias, and acute lung injury similar to a severe presentation of COVID-19; and (3) hypersensitivity reactions [60]. The specificity of antibodies is likely to give rise to idiosyncratic side effect profiles and not all antibodies will cause the same degree of immunosuppression or immune cell activation.

We expect humanized antibodies to be less likely to cause serum sickness than antibodies raised in horse (i.e., Anavip) or raised in sheep (i.e., CroFab). Anavip (Rare Disease Therapeutics, Inc.) and CroFab (Protherics Inc.) are antibody therapies used to treat envenomation by North American crotalids. A humanized antibody is an antibody raised from non-human species and then modified to increase its similarity to naturally occurring human antibodies so as to provoke less of a humoral immune response in humans. For example, antibodies raised in mice can have their Fc region replaced by human sequences to decrease immunogenicity [61].

Viral Replication Inhibitors

An RNA virus must co-opt the host machinery for translating RNA into protein. As discussed in "Biology of Coronavirus" section, the genomes of coronaviruses are sequences of

nucleotides that human cells treat, effectively, as mRNA. To produce virions, a virus must make a copy of its RNA genome using viral RNA-dependent polymerase and synthesize all the proteins needed to form the virion, including the capsid and spike proteins.

Nucleotide Analogs

The overall goal of nucleotide analogs is to halt the production of new viral RNA, making it impossible for infected host cells to become manufacturing sites for new virions. Nucleotide analogs do this by incorporating a base into the replicating strand from which viral RNA polymerase cannot elongate.

Remdesivir and Favipiravir

Remdesivir (Gilead) is a prodrug metabolized to an adenosine nucleotide analog. It has demonstrated in vitro efficacy against SARS-CoV-2 [21]. Favipiravir (Toyama Chemical) mimics adenosine and guanine leading RNA-dependent RNA polymerase to make error-ridden nonfunctional viral genome progeny [62].

In the absence of data on remdesivir or favipiravir toxicity, one can extrapolate from the toxicity reported for other nucleoside analogs. Metabolic acidosis can occur with therapeutic use, usually after a month or more of treatment [63], though it has also been observed in acute overdose. Severe metabolic acidosis with elevated lactate is a toxicity of nucleoside analogs that has a high mortality. Peripheral neuropathy is a dose-related, subacute phenomenon seen with stavudine, 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T), didanosine (ddI), and zalcitabine (ddC) [64]. Bone marrow suppression is seen in about 5% of patients starting 3′-azido-2′,3′-dideoxythymidine (AZT) treatment [65]. Pancreatitis and myopathy have been observed with several medications in this class [66]. Each of these toxicities are thought to reflect mitochondrial dysfunction. In addition to inhibiting viral polymerase, nucleoside analogs also inhibit mitochondrial DNA polymerase-gamma, leading to decreased mitochondrial DNA and synthesis of mitochondrial proteins [64]. If severe toxicity occurs, the offending agent should be stopped. There is no specific antidote, though some suggest that treatment with mitochondrial cofactors, such as thiamine, riboflavin, L-carnitine, and vitamin C, may help patients recover [63]. Additional idiosyncratic toxicities may also occur, for example, tenofovir inducing crystalluria [67].

Protease Inhibitors

Protease inhibitors prevent infected cells from forming competent new virions by binding to and inactivating viral proteases to halt viral replication. Viral proteases process the initial products of translation to make the final versions of viral proteins. Viral proteases are unique to each virus. The crystal structure of the SARS-CoV-2 main protease has recently been identified [68]. Drugs that are effective against one virus are not expected to be effective against another virus unless the viruses' proteases are sufficiently similar. Protease inhibitors that target HIV protease, for example, do not target hepatitis C protease, even though both are RNA viruses. The crystal structure of the SARS-CoV-2 main protease has been identified and it closely resembles SARS-CoV-1 but not any known human proteases [68].

Lopinavir-Ritonavir

Screening of HIV protease inhibitors against SARS-CoV-1 found that nelfinavir, but not ritonavir, inhibited viral replication in vitro [69]. Although this study described the efficacy of nelfinavir, it did not determine if nelfinavir's effects were specifically due to SARS-CoV-1 protease inhibition. Another screening study identified lopinavir, but not nelfinavir, as inhibiting SARS-CoV-1 and demonstrated that treatment with lopinavir-ritonavir (Abbvie) and ribavirin reduced mortality and ARDS compared to treatment with ribavirin alone [70]. Ritonavir is added to lopinavir as a pharmacokinetic booster [71]; ritonavir is a potent inhibitor of cytochrome CYP 3A4, the enzyme that inactivates lopinavir. The same regimen (lopinavir-ritonavir with ribavirin) was used as post-exposure prophylaxis for healthcare workers treating patients with severe Middle East Respiratory Syndrome (MERS, also caused by a coronavirus), which showed a 40% decreased risk of infection, and no severe adverse effects were reported [72].

Early experience in the treatment of SARS-CoV-2 suggests that patients treated with lopinavir-ritonavir commonly experience nausea and vomiting as well as mild transaminase elevations [73]. Lopinavir-ritonavir seemed to hasten recovery in a case series of 10 hospitalized patients [74], but a larger randomized trial of 99 test subjects and 100 controls showed no difference in time to clinical improvement [75]. In all three studies, many patients receiving the combination left the study due to nausea, vomiting, and diarrhea.

Acute overdose of protease inhibitors is uncommon, but a large overdose of more than 50 g of lopinavir-ritonavir was generally well tolerated and managed supportively [76]. Ritonavir is a very potent inhibitor of CYP3A4 (Ki 0.59 ± 0.12μM), and may slow the metabolism of drugs which are substrates of CYP3A4, leading to potential dangerous drug-drug interactions [77].

Lopinavir-ritonavir is associated with increases in transaminases in 3–10% of individuals being treated for HIV, though clinically significant liver injury appears to be rare and resolves with drug removal [78]. A cholestatic pattern of injury is sometimes observed, which may be explained by lopinavir reducing expression of the bile salt export pump (BSEP) in human hepatocytes [79]. A latency period of 1 month or longer is common,

which may make this a less relevant feature in the treatment of SARS-CoV-2.. There are genetic variations (polymorphisms) across patients, such as a loss-of-function of CYP3A4 [80], that can make patients more susceptible to metabolic toxicities [81]. Screening for these susceptibilities is not practical in the setting of short duration treatment for SARS-CoV-2. Arthralgias may occur in 35% of patients [82]. Achilles tendinopathy has been reported in individuals taking lopinavir-ritonavir, even in short-term use for post-exposure prophylaxis [83].

Protease inhibitors cause lipodystrophy, characterized by central adiposity, dorsocervical fat deposition ("buffalo-hump"), and extremity wasting when used to treat HIV. Multiple mechanisms may explain these metabolic derangements, including inhibition of SREBP-1, a master regulator of lipogenesis and adipocyte differentiation, and direct inhibition of glucose transporter-4 [79].

Vaccines

The goal of a vaccine is to develop longstanding immunity without exposure to the full brunt of the disease. In vivo and phase I trials of new vaccine candidates are currently underway to evaluate safety and immune response.

S-trimer

S-trimer refers to a vaccine made to resemble the spike protein, the protein on the capsid surface that binds to ACE2 in alveolar cells to initiate viral entry. The spike protein is an attractive target because it elicits an immune response and mutations in the spike protein may explain the virulence of SARS-CoV-2. The SARS-CoV-2 spike protein binds about ten times more tightly to ACE2 than the SARS-CoV-1 spike protein does [84]. The mutations also create the need for new therapeutics as that same paper noted that three monoclonal antibodies raised against SARS-CoV-1 spike protein did not strongly bind to SARS-CoV-2 spike protein. The S-trimer vaccine candidate is expected to be tested in pre-clinical studies shortly. The generation of antibodies against the spike proteins of SARS or MERS [85] was challenging because the spike proteins of coronaviruses are heavily glycosylated.

Nucleic Acid Vaccines

DNA vaccines work by inserting DNA plasmids that encode antigens into host cells. This generates cellular and humoral antigen-specific immunity, allowing the patient to mount an immune response to the target disease [86, 87]. Studies are ongoing regarding the effectiveness and longevity of vaccine immunogenicity for several infections, including HIV, MERS, Ebola, and Zika.

GLS-5300 (Inovio Pharmaceuticals, Inc., GeneOne Life Science Inc.), a DNA vaccine for MERS-CoV, is entering phase 2 trials. MERS-CoV is the coronavirus that causes Middle East respiratory syndrome. GLS-3500 encodes for the MERS-CoV spike glycoprotein and demonstrated an immunogenic response in non-human primates. In phase 1 studies, GLS-5300 demonstrated local injection site symptoms in most patients, along with headaches, malaise, and myalgia. Some patients developed mild creatinine phosphokinase elevations, but no renal injury or myopathy developed [88].

INO-4800 (Inovio Pharmaceuticals, Inc.) is a DNA vaccine that encodes the SARS-CoV-2 spike protein and is delivered intradermally [89]. A phase 1 clinical trial INO-4800 started in early April. To enhance uptake, DNA vaccines are administered via an electroporation device such as the CELLECTRA [90]. Safety and tolerability studies of the CELLECTRA did not identify severe adverse events, though injection site pain, mild elevations in creatinine phosphokinase, and paresthesias were described [91]. Animals that received the vaccine produced antibodies and T cells against the virus [13]. A phase 1 clinical trial INO-4800 has started.

Beyond local tissue reactions, DNA vaccines raise the concern of adverse events from plasmid integration with host DNA, disrupting usual transcription. In vitro studies thus far suggest that the rate of insertional mutagenesis is lower than the rate of spontaneous mutations in mammalian cells. Generally, DNA vaccines appear to be safe, without apparent off-target effects or idiosyncratic toxicities [87].

mRNA Vaccines

Messenger RNA (mRNA) vaccines direct the production of antigens but, unlike DNA vaccines, cannot integrate into the host genome, lowering the risk of mutations [92]. Once in the cytosol, mRNA vaccines direct antigen production and then rapidly degrade. Moderna Therapeutics, in collaboration with the National Institute of Allergy and Infectious Disease, announced that it launched a phase I trial of the mRNA-1273. The mRNA-1273 vaccine is a novel lipid nanoparticle encapsulated mRNA-based vaccine that encodes for the prefusion stabilized form of the spike protein [93]. The prefusion form refers to the spatial conformation of the spike protein before it binds to ACE2. An antibody against the prefusion form could prevent viral entry as well as mitigate the spread of virions.

Miscellaneous

Azithromycin

Azithromycin is a macrolide antibiotic used for its ability to inhibit bacterial protein synthesis by binding to the 50S subunit of the bacterial ribosome. It is also used to treat exacerbations

of COPD and reactive airway disease. This second use may reflect azithromycin's modulatory effect on immune cells. It reduces respiratory syncytial virus (RSV) release by decreasing interferon signaling in vivo and inhibits proinflammatory cytokine release in airway smooth muscle and epithelial cells [94]. A prospective trial in France of 22 patients noted that a combination of 600 mg hydroxychloroquine and azithromycin (500 mg on the first day and then 250 mg each day for the next 4 days) reduced viral load more effectively than hydroxychloroquine alone [95]. This trial broke randomization as those who declined treatment were analyzed as controls.

The main toxicity from azithromycin is QTc prolongation leading to cardiac dysrhythmias owing to hERG (an inward rectifying potassium channel) blockade [96]. In one cohort study, a 5-day course of azithromycin led to a nearly threefold increase in cardiovascular death as compared to other antibiotics [97], although a follow-up cohort study of similar size (2,204,100 prescriptions) [98] and meta-analysis of prospective randomized controlled trials identified no increased risk in mortality [99].

We expect the combination of azithromycin and chloroquine or hydroxychloroquine to be more likely to precipitate cardiac dysrhythmias than either alone. Azithromycin weakly blocks hERG from conducting potassium ions across the cardiac membrane while chloroquine and hydroxychloroquine decrease the number of ions available for transit.

ACE Inhibitors and Ibuprofen

There is concern in the media that angiotensin-converting enzyme (ACE) inhibitors may increase susceptibility to SARS-CoV-2 [100]. ACE inhibitors can increase ACE2 expression in human tissue [101], potentially creating more binding sites for SARS-CoV-2. The receptor-binding domain (RBD) of SARS-CoV-2 has a high affinity for ACE2. However, ACE inhibitors have been shown to reduce viral entry by competitive inhibition of spike protein binding to ACE2 in vitro [102]. Lastly, alveolar cells infected with coronavirus express less ACE2 on their cell surface than normal cells in vitro. Knocking down the expression of ACE2 in uninfected mice creates acute lung injury histologically similar to that seen in SARS, suggesting intact ACE2 function serves a lung-protective role [103].

One could interpret these findings to suggest that those patients taking ACE inhibitors may benefit from stopping them, while those not taking them may benefit from starting. At this time, however, there is no direct clinical evidence of any impact of ACE inhibitors on the clinical trajectory of those with COVID-19. A retrospective study from Wuhan, China identified hypertension, diabetes, and cerebrovascular disease as poor prognostic factors, but did not isolate the relative risk attributable to taking an ACE inhibitor [1]. The worse outcome in this study of patients on ACE inhibitors may reflect the generally poor outcomes of patients with multiple comorbidities. No patient should abruptly discontinue an ACE inhibitor except at the direction of a physician. Blood pressure rapidly rises in the first 48 hours after ACE inhibitor discontinuation [104], which could precipitate hypertensive emergencies and subsequent acute pulmonary edema.

That same study stated that ibuprofen increases ACE2 receptor expression but provided no supporting evidence. Neither the WHO [105] nor the FDA [106] recommends withholding ibuprofen for symptomatic treatment of COVID-19.

Convalescent Plasma

Convalescent plasma refers to pooled plasma or immunoglobulins from patients who have been infected and then recovered from a disease. In ten patients seropositive for SARS-CoV-2 and hypoxic, but not intubated, one dose of 200 mL of convalescent plasma led to a nearly immediately undetectable viral load and improved oxygenation in 3 days [107]. In five patients who were intubated with radiographic evidence of acute lung injury, transfusion of 200–250 mL of convalescent plasma on days 10 and 22 of admission reduced hypoxia in all five and three were able to be weaned from mechanical ventilation [108].

Risks commonly associated with plasma transfusion include (1) transfusion-associated acute lung injury (TRALI), (2) transfusion-associated circulatory overload (TACO), and (3) allergic/anaphylactic reactions. Other less common risks include (1) transmission of infections, (2) febrile non-hemolytic transfusion reactions, (3) RBC alloimmunization, and (4) hemolytic transfusion reactions [109]. A meta-analysis of studies that used convalescent plasma to treat SARS and influenza A (H1N1) reported no adverse effects beyond minor infusion reactions such as chills and fevers [110]. Four critically ill patients with SARS-CoV-2 had no significant adverse events when treated with convalescent plasma and supportive care [111].

Conclusion

The unmet need for effective treatments for COVID-19 has spurred pharmaceutical companies to develop or repurpose therapeutics against SARS-CoV-2. Frontline providers may be unfamiliar with the usage of older therapeutics, such as chloroquine, or medicines typically used by subspecialists, like DNA vaccines, protease inhibitors, or convalescent plasma therapy. In conjunction with effective infection control policies, a combination of measured and reasoned repurposing of existing therapies, development and vetting of novel drugs, and guidance by medical toxicologists may reduce morbidity and mortality from COVID-19 and avoid cases where the cure is worse than the disease.

Acknowledgments We thank Dr. Lydia Bunker for reviewing the manuscript, useful discussions, and edifying comments.

Sources of Funding Dr. Michael A. Chary is supported by the Loan Repayment Program (National Institute on Drug Abuse, National Institutes of Health). Dr. Sudeh Izadmehr is supported by the Loan Repayment Program (National Center for Advancing Translational Sciences, National Institutes of Health).

Compliance with Ethical Standards

Conflict of Interest Dr. Burns is the Pediatric Toxicology Section Editor for *UpToDate*. No other authors report conflicts of interest.

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