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Editorial

Doxycycline as a potential partner of COVID-19 therapies

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is a major public health challenge, and the current antiviral arsenal for treatment is limited, with questionable efficacy. Major efforts are under way for discovery of new effective agents, but the validation of new potential treatments for COVID-19 may take a long time. Therefore, the repurposing of existing drugs for new indications is needed. In this article, we argue for the potential benefits of using doxycycline with either hydroxycholoroquine or other putative agents for COVID-19 treatment, as doxycycline has antiviral and anti-inflammatory activities by dampening the cytokine storm and to prevent lung damage.

Introduction

The 2019 novel coronavirus infection, dubbed COVID-19, has created an unprecedented public health crisis and threatened the lives of millions of people worldwide [1]. The immunopathogenesis of severe COVID-19 is partially understood and it is likely involves both a virus-driven damage and an exuberant host inflammatory response, both contributing to acute lung injury, acute respiratory distress syndrome (ARDS), and multiple organ failure [2]. Since there is no clear evidence of efficacy among available antivirals for COVID-19 and since the discovery and clinical testing of novel antiviral agents takes a long time, repurposing of existing drugs is of paramount importance. Priority should be given to drugs that combine antiviral and anti-inflammatory effects. In addition, candidate drugs should have an acceptable tolerability profile with no major adverse events or toxic effects.

Hydroxychloroquine with or without azithromycin

Several agents, including hydroxychloroquine have purported in vitro activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19 [3]. The anti-viral mechanism of hydroxychloroquine has not been entirely elucidated, but likely occurs through a change in pH at the surface of the cell membrane that inhibits the fusion and assembly of the virus [3]. Recent uncontrolled clinical data by Gautret et al. demonstrated that hydroxychloroquine treatment is associated with rapid virologic clearance, which occurred at day 6 in 70 % of patients on monotherapy versus in 12.5 % of patients on supportive care; adding azithromycin slightly enhanced this effect, with virologic clearance occurring at day 5 in 100 % of patients given the combination [4]. Despite the notable limitations of that study, including small number of patients, absence of randomization, selection bias for controls, and use of treatment in less-ill patients, this antimicrobial combination has generated a great deal of interest [4].

However, the hydroxychloroquine and azithromycin combination has raised major safety concerns, specifically, drug-drug interactions and cardiotoxicity, including fatal arrhythmia, particularly among infected elderly patients with underlying cardiopulmonary chronic illness [5]. The heightened risk of cardiotoxicity among older patients is of particular concern [6]. Also, patients with COVID-19 may develop infection-related cardiomyopathy (frequency is unknown) with direct and indirect cardiovascular complications, including acute myocardial injury, fulminant myocarditis (with a mortality rate up to 40 %–70 %), arrhythmias, and venous thromboembolism [5,7–9].

Antiviral effects of doxycycline

Given the risks of hydroxychloroquine and azithromycin in combination, we suggest hydroxychloroquine with doxycycline as a better alternative to azithromycin. Doxycycline and other tetracycline derivatives such as minocycline exhibit anti-inflammatory effects along with *in vitro* antiviral activity against several RNA viruses. Use of these agents have been associated with clinical improvement, even reversal of cytokine storm in some infections caused by RNA viruses, such as dengue fever [10].

The mechanism of the antiviral effects of tetracycline derivatives may be secondary to transcriptional upregulation of intracellular zinc finger antiviral protein (ZAP), an encoding gene in host cells [11,12]. ZAP can also bind to specific target viral mRNAs and represses the RNAs translation [13,14]. Experimental studies have used tetracycline to induce the overexpression of host ZAP in HEK293, rats and monkeys cell lines (Vero cells), which contributed to inhibition of RNA viruses such as the Dengue, Ebola, Human Immunodeficiency Virus, Zika, and Influenza A viruses [11,12,15–18].

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Also, in vitro studies have showed that doxycycline can repress Dengue virus infection in Vero cells through the inhibition of dengue serine protease enzymes and of viral entry [17,19]. Doxycycline showed the capacity to inhibit dengue virus replication in Vero cells culture and likely it interacts with the dengue virus E protein that is required for virus entry [19]. Similarly, doxycycline controls Chikungunya virus (CHIKV) infection through the inhibition of CHIKV cysteine protease of Vero cells and showed significant reduction of CHIKV blood titer of mice [20].

In addition, tetracycline derivatives such as doxycycline are highly lipophilic antimicrobials that chelate zinc compounds on matrix metalloproteinases (MMPs) of mammalian cells [21], and an in vitro study showed that murine coronaviruses rely on MMPs for cell fusion and viral replication [22]. Other mechanisms of viral fusion and replication by coronaviruses utilize host proteases [22], could be a possible target to doxycycline.

Anti-inflammatory effects of doxycycline

In COVID-19, elevated levels of blood interleukin (IL)-6 have been more commonly observed in severe COVID-19 illness and among non-survivors, suggesting that mortality might be due to virally-driven hyperinflammation and to cytokine storm [23]. Intense proinflammatory state has a central role in the pathogenesis of dengue and hemorrhagic fever, leading to cytokine storm [24]. Importantly, doxycycline reduced pro-inflammatory cytokines, including IL-6 and tumor necrosis factor (TNF)-α, in patients with dengue hemorrhagic fever, and the mortality rate was 46 % lower in the doxycycline-treated group (11.2 %) than in the untreated group (20.9 %) [24]. Moreover, doxycycline was more effective than tetracycline in the reduction of these pro-inflammatory cytokines [25].

Similarly, an in vitro study suggested that treatment with minocycline had dual anti-inflammatory effects and viral replication in cells infected with Enterovirus 71 infection, as minocycline reduced the viral cytopathic effect, viral protein expression, viral titers, levels of IL-6 and IL-8, and relative mRNA expression of TNF-α. Also, in a murine model, minocycline inhibited IL-6 and granulocyte colony-stimulating factor in plasma and TNF-α in the cerebellum [26].

In addition, severe acute respiratory syndrome–related coronavirus (SARS-CoV) encompasses a papain-like protease that significantly triggers an early growth response protein 1 (Egr-1)–dependent activation of transforming growth factor beta 1 (TGF-β1), resulting in upregulation of pro-fibrotic responses in vitro and in vivo in the lungs [27,28]. Recent computational methods study identified doxycycline among the drugs that could potentially be used to inhibit SARS-CoV-2 papain-like protease [29].

Severe COVID-19, ARDS, and pathophysiologic and therapeutic considerations

Respiratory failure from ARDS is the leading cause of mortality in COVID-19 patients [30]. Various pro-inflammatory cytokines and chemokines, including IL-6, TNF-α, and profibrotic factors (*TGFB1, CCN2,* and *PDGFA*), are also implicated in tissue damage and vascular leakage and can stimulate pulmonary fibrosis in SARS-CoV infection [31]. The pathologic features of COVID-19 closely resemble those of SARS-CoV infection, which causes massive lung tissue remodeling through the urokinase, coagulation, and wound-healing pathways and through extracellular matrix proteins, including MMPs [31]. MMPs are involved in lung remodeling and destruction of the extracellular matrix, leading to damage of the endothelial basal lamina and increased vascular permeability [32,33]. Importantly, mechanical ventilation, which has a primary role in ARDS management, is associated with further lung injury through activation of MMPs, leading to ventilation-induced lung injury [34].

As mentioned earlier, doxycycline is a strong and broad-spectrum inhibitor of MMPs (a family of more than 24 zinc-dependent proteases). Furthermore, experimental studies have showed that treatment with doxycycline conferred a protective role in lung injury [35,36]. A prophylactic use of doxycycline in mice infected with virulent influenza H3N2 virus attenuates the occurrence of acute lung injury [37]. The tetracycline class of antimicrobials overall has proven a clinically useful tool in MMP inhibition through their ability to chelate the catalytic Zn^{2+} ion, which is essential for MMP activity, independently of their antimicrobial properties [34]. Among the tetracycline derivatives, doxycycline is the most potent MMP inhibitor, even at a subantimicrobial dose (25 mg) [34]. As lung immune injury/ARDS is prominent in patients with severe COVID-19, inhibiting MMPs may help repair the damaged lung tissue and enhance recovery [38].

Future study design considerations

In light of these potential benefits, we propose the use of doxycycline (preferably) or minocycline as a partner agent with hydroxychloroquine or with other promising antiviral COVID-19 therapies such as remdesivir, particularly in elderly patients with multiple health conditions, especially cardiac comorbidities. Doxycycline is usually prescribed as a part of empiric treatment for atypical bacterial pneumonia or community-acquired pneumonia based on the recent evidence-based clinical practice guidelines [39]. Therefore, it may be useful to conduct a large retrospective cohort study assessing disease severity, co-infections, mortality rate, length of hospitalization, and the need for invasive ventilation among COVID-19–infected patients who received a doxycycline-based or other tetracycline-based empiric antimicrobial regimen.

In addition, placebo-controlled randomized clinical trials divided into two arms (treatment arm, antiviral (i.e. remdesivir) plus doxycycline versus the control arm, antiviral plus placebo) and should enroll COVID-19 patients regardless of the severity of illness at presentation. Primary end points, in addition to mortality rate, should include clinical improvement (defervescence), progression to respiratory failure, need for mechanical ventilation (duration and extubation), virologic clearance, and length of stay at the hospital.

Conclusion

Because patients with COVID-19 are in need of both antiviral and anti-inflammatory treatment as well as protection against lung damage, studies of proposed combination therapy is warranted. As doxycycline is inexpensive and widely available, has a safe tolerability profile, and is an attractive option for the treatment of COVID-19 as well as potentially alleviating the lung sequelae and also providing coverage against atypical bacterial pneumonia such as *Mycoplasma* pneumoniae and *Legionella pneumophilia*.

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Alexandre E. Malek: Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Bruno P. Granwehr:** Writing - original draft. **Dimitrios P. Kontoyiannis:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

None of the authors have any financial or personal relationships with other people or organizations that might pose a conflict of interest in connection with the submitted article.

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