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REVIEW ARTICLE

Possible Role of Tetracyclines on COVID-19: Recycling Well-Known Old Drugs from the Shelf

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Abstract

We are in the midst of a pandemic due to the novel coronavirus SARS-CoV-2. Innovative therapies are in the lookup around the world. Recently, chloroquine and hydroxychloroquine in addition to Azithromycin were proposed to be used in patients with severe disease even though strong evidence is lacking. We propose the use of tetracyclines in addition to anti-virals early in the curse of the disease in order to prevent the Cytokine Storm Syndrome associated with COVID-19 and prevent ARDS. The proposed mechanisms of tetracyclines are: 1) anti-apoptotic properties; 2) decrease the Myeloperoxidase and ROS releaser from immune cells; 3) decrease neutrophil and monocyte migration and chemotaxis; 4) decrease the secretion of pro-inflammatory and vasoactive cytokines from macrophages (IL-1 beta, IL-8, and TNF-alpha); 5) inhibition of iNOS expression; 6) inhibition of chemotaxis of peripheral monocytes; 7) inhibition of IL-6 production and its receptor system; 8) prevention of fibrosis; and 9) inhibition of metalloproteinases (particularly MMP-2 and 9). Tetracyclines are well-known drugs with lower costs, and are not associated with adverse effects like QT prolongation. Clinical trials are needed to test our hypothesis.

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Introduction

We are in the midst of a pandemic caused by the novel coronavirus SARS-CoV-2 (named by WHO on Feb 11, 2020). [1] A recent Morbidity and Mortality Weekly Report (MMWR) revealed that among 44 cases with known outcome, 15 (34%) deaths were reported among adults aged ≥85 years, 20 (46%) among adults aged 65–84 years, and nine (20%) among adults aged 20–64 years. [2] Case-fatality percentages increased with increasing age, from no deaths reported among persons aged ≤19 years to highest percentages (10%–27%) among adults aged ≥85 years. Some reports showed deaths in the group of < 19 years of age, but it is rare. The number of cases is trending up globally and healthcare systems are being overwhelmed in certain regions. Of note, the mortality is significantly higher than with seasonal Influenza. [1,2] Innovative therapies are in the lookup around the world in international academic centers until an effective and safe vaccine is available. Recently, Chloroquine (CQ) and Hydroxychloroquine (HCQ) were proposed to be used in COVID-19 patients with the goal of decreasing mortality after some positive results in a study in France where a rapid fall of nasopharyngeal viral loads tested by qPCR was noted, with 83% negative at Day7, and 93 % at Day 8. [3] The number of patients presumably contagious (with a PCR Ct value <34) steadily decreased over time and reached zero on Day 12. [4] The FDA approved an Emergency Use Authorization (EUA) of this drug under the directives of the President. On April 4, 2020, the FDA recommended against the combination of CQ or HCQ with Azithromycin outside clinical trials and in the outpatient settings due to lack of convincing clinical evidence of its effectivity and serious concerns about cardiac toxicity like QT prolongation when used in combination. [5]

The FDA also issued an EUA on May 5, 2020 for the investigational antiviral drug remdesivir for the treatment of suspected or laboratory-confirmed COVID-19 in adults and children hospitalized with severe disease. [6]

The most common cause of mortality due SARS-CoV-2 (as well as with SARS-CoV-1 and MERS-CoV) is Acute Respiratory Distress Syndrome (ARDS). [1, 2, 7, 8] The objective of this review is to explore the possible role of tetracyclines on decreasing the mortality observed with SARS-CoV-2 as an immunomodulator targeting the COVID-19-associated-CSS/ARDS. We extrapolated data from the previous outbreak of SARS-CoV-1, for which there is extensive documentation and is phylogenetically related to COVID-19.

Pathogenesis and Histopathology of SARS-CoV-1 and SARS-CoV-2. Similarities With Influenza-Associated-ARDS

Some common features were seen in the histopathology of SARS-CoV-1, MERS, and SARS-CoV-2). [1, 7] We will call these 3 related viral pathogens Highly Pathogenic Human Coronavirus (HPHCoV). COVID-19 is a systemic disease since ACE-2 receptors (SARS-CoV-2's receptor) are located in multiple tissues including lung epithelial lining, vascular endothelial cells (arterial and venous), and the mucosal cells of the intestines, epithelial cells of the renal tubules, cerebral neurons and immune cells. [9] The main target of SARS-CoV-2, however, is the lung, which is driving mortality. Due to the paucity of data from COVID-19 autopsies at the time of preparation of this manuscript, we will describe mainly the histopathology findings seen during the SARS-CoV-1 outbreak in 2002 for which there is much more data.

In the lung tissue from autopsies of HPHCoV usually there are extensive hyaline membrane formation, interstitial infiltration with lymphocytes and mononuclear cells, Giant cells with markers of macrophages, and desquamation of pneumocytes in the alveolar space. Macroscopically patchy areas of lung consolidation and edema, pleural effusions, focal hemorrhages, and mucopurulent material in the tracheobronchial tree are usually seen. Diffuse alveolar damage (DAD) is common being septal and alveolar fibrosis expectable in later stages. [8] Microscopically, viral particles can be seen not only in type 1 and 2 pneumocytes [7] but also on the airway and alveolar epithelial cells, vascular endothelial cells, monocytes and lymphocytes and macrophages. [8] Multiple cellular infiltrates were observed including neutrophils and macrophages (predominance of activated macrophages). The peripheral cell count usually shows lymphopenia, which may reflect active lymphocytic recruitment to the lungs, induced-apoptosis, or selective suppression of lymphocytes precursors in the bone marrow. Activated monocytes and lymphocytes in the lung tissue may recruit, orchestrate, and exacerbate the inflammatory activity of neutrophils, showing a connection between adaptive and innate immune responses. In animal models of SARS-CoV-1, there is initially a massive recruitment of pathogenic inflammatory monocyte-macrophages (IMMs), which cause further self-accumulation and local release of TNF, IL-6, IL1-β, and iNOS. [8] IMMs induce lymphocyte apoptosis with decreased viral clearance and promotion of local viral persistence. SARS-CoV-1 infection of Dendritic cells (DCs) and macrophages induces up-regulation of pro-inflammatory cytokines like TNF, IL-6, and a significant up-regulation of inflammatory chemokines CCL3, CCL5, CCL2, and CXCL10. The elevation of Interferon is mild which impairs viral clearance (evasion of innate immune system). Severe SARS-CoV-1 patients had significantly higher serum levels of pro-inflammatory cytokines (IFN-γ, IL-1, IL-6, IL-12, and TGFβ) and chemokines (CCL2, CXCL10, CXCL9, and IL-8) compared with SARS-CoV-1 patients with mild disease. [8] Interestingly, in very sick patients the anti-inflammatory cytokine IL-10 was decreased. There are no studies showing the role of metalloproteinase in SARS-CoV-1/2 associated-ARDS. All the above can explain the Cytokine Storm Syndrome (CSS) documented in severe cases of SARS-CoV-1, which also applies to COVID-19 cases.

In summary, there is evidence showing a dysregulated immune response with an exuberant inflammatory disease, evasion of the immune response (through inhibition of Interferon molecules), and disseminated direct cytopathic effects. [8] ARDS is a common final event that leads to death. Interestingly, the ARDS seems to occur once the viral titers drop, which means that most of the tissue damage is probably immune-mediated. It looks like monocytes-macrophages and neutrophils are the ultimate culprit that drive the local inflammatory cytokine storm and tissue damage.

The consequences of the overwhelming immune response are: 1) Epithelial and endothelial apoptosis with vascular leakage; 2) Decreased SARS-CoV-1/2 specific T cell immune responses due to the exuberant immune response with T cell apoptosis (and high titer viral replication); and 3) Persistent activation of macrophages, neutrophils, and fibroblasts (with lung fibrosis).

Analogy with Influenza-Associated-ARDS

The mortality associated with Influenza pneumonia is also attributed to ARDS. [10] Making an analogy of a possible common mechanism of lung-tissue damage between HPHCoVs and influenza brings up the possible role of metalloproteinases ("Gelatinasas"). During Influenza pneumonia, histopathological features revealed a prominent neutrophilic infiltration within the affected areas implicating their primary role in lung injury. These activated neutrophils cause local release of toxic granular products such as matrix metalloproteinases (MMPs), myeloperoxidase (MPO), elastase, and reactive oxygen intermediates (ROI) contributing to lung injury. Gelatinases (including MMP-2 and MMP-9), which are zinc-dependent endopeptidases, affects the components of the basement membranes such as gelatin and collagen IV, and the epithelium and endothelium in the alveolar-capillary barrier. The above mechanism accounts for the intense increased permeability and exudation into the alveolar space seen during ARDS with thickening of the alveolar-arterial space and refractory hypoxemia. There may be multiple similarities between some final events that leads to death between HPHCoVs and severe influenza pneumonia since in both there is an intense lung infiltration of macrophages-neutrophils, which may release MMPs along with ROS.

Tetracyclines, the Molecule: Anti-Inflammatory Properties and its Possible Role in SARS-CoV-2-associated-ARDS

Our group performed an extensive review of tetracyclines when we proposed to use this molecule to decrease the accelerated aging process of well-controlled HIV patients of ART. [11] We will review their anti-inflammatory and immunomodulatory properties of this molecules applied to HPHCoV models.

First discovered in the 1950s by screening organisms obtained from the soil while looking for new antibiotics (Streptomyces aureofaciens produced chlortetracycline, trade name Aureomycin), it was clear that tetracyclines not only had anti-infective but also anti-inflammatory properties as well. Since then, tetracyclines (especially the new second generation's tetracyclines like doxycycline and minocycline) have been extensively used for non-infectious disease processes such as rosacea, bullous dermatoses, neutrophilic diseases, pyoderma gangrenosum, sarcoidosis, aortic aneurysms, cancer metastasis, periodontitis; autoimmune diseases such as rheumatoid arthritis, hidradenitis suppurativa, and scleroderma; and COPD. [12-26]

The anti-inflammatory properties of Tetracyclines are (**Table 1**):

1. It was shown that tetracyclines have anti-apoptotic properties because they inhibit the caspase 1 and 3 pathways along with decreasing the cytochrome c release from the mitochondria. [16] This is important since T-cell CD4+ cell lymphopenia was seen in COVID-19 patients. Decreasing the apoptosis of lymphocytes is extremely important to mount an early specific CD4 and CD8 immune response against SARS-CoV-2 infected cells to control the initial high viral replication and avoid local viral persistence. The decrease in apoptosis may also involve the T-cell suppressor subtype, which may help control the exuberant immune activation. The suppression of apoptosis would also prevent the epithelial and endothelial induced apoptosis, which exacerbates vascular leakage, micro thrombosis, and further inflammation-exudation to the alveolar and vascular space.
2. On in vitro models in rats, tetracyclines not only decreased the Myeloperoxidase (MPO) release from neutrophils but the neutrophil migration as well. [27] Of note, the prolonged MPO persistence on tissues may predispose to local oxidative stress and, hence, extend the local inflammatory process even further. The same group described that minocycline and doxycycline also decrease the carrageenan-induced paw edema in rat models, which may be related to the fact that tetracyclines decrease the secretion of pro-inflammatory and vasoactive cytokines reducing the increase permeability associated with acute and chronic inflammation. On the same animal models, tetracyclines showed not only antioxidant properties but inhibition of iNOS expression as well. [26] Decreasing chemotaxis, secretion of pro-inflammatory cytokines, vascular leakage, and local oxidative stress could be extremely important to prevent COVID-19-associated-ARDS/CSS. [10]
3. A classic effect of these classes of molecules is that they decrease the secretion of pro-inflammatory cytokines from macrophages (IL-1 beta, IL-8, and TNF-alpha) in response to stimulation with LPS, which points towards an important inhibitory mechanism on the innate immune system. In postmenopausal women with periodontitis, low-dose doxycycline lowered markers of systemic inflammation such as hs-CRP, myeloperoxidase (MPO), MMP-8, TIMP-1, MMP-9, MMP-2, IL-6, TNF-a, and IL-1b. [14, 28] In a SIV model, minocycline treatment decreased the pro-inflammatory CD14+CD16+ monocytes, and reduced their expression of CD11b, CD163, CD64, CCR2 and HLA-DR along with a decrease of IL-6 production by monocytes following LPS stimulation. [15] Tetracyclines also inhibit the chemotaxis of peripheral monocytes to the activated endothelium since the production of vascular endothelial growth factor (VEGF), and hence, of MCP-1, is decreased. [29, 30] It has been shown that minocycline not only suppress IL-6 production, but also its receptor system and signaling pathways. [31] Tetracyclines also suppress hydrolases, such as phospholipase A2, which is an important enzyme that mediates activation of inflammatory mediators such as prostaglandins. [32] Since COVID-19-associated-ARDS is driven by an exuberant immune response (CSS) orchestrated by recruited activated monocytes and neutrophils, doxycycline could be important in controlling the local over-activation of the innate immune system. In addition, decreasing the production of TNF by activated macrophages may decrease the apoptosis of lymphocytes which looks like is TNF-driven. [8] Of note, inhibition of IL-6 is the mechanism of action of tocilizumab, which is being evaluated currently in clinical trials for COVID-19.
4. Fibrosis is the culmination of many types of chronic inflammatory processes and it can be either beneficial or

Table 1. Characteristics of Tetracyclines and Interruption of inflammatory Pathways.

Properties Tetracyclines	Consequences
Anti-apoptotic properties	• Increase lymphocyte count (lymphocytes) • Decrease vascular leakage (endothelium) • Decrease alveolar exudation (epithelium)
Decrease Chemotaxis and Migration of Innate Immune cells	• Decreased local release of MPO, ROS, and MMP
Decreased Secretion of Pro-inflammatory Cytokines from Activated Macrophages	• Decrease of immune-activation • Decreased Recruitment of Immune cells • Decreased Duration and Intensity of the CSS • Decreased ARDS
Inhibition of IL-6 and its Pathway	• Highly involved on CSS (Tocilizumab)
Inhibition of MMP 2/9	• Decreased degradation of alveolar-capillary membrane • Decreased alveolar exudation and leakage • Improve lung histology
Anti-fibrotic Properties	• Decrease the progression to lung fibrosis • Decrease sequela

harmful. Excessive fibrosis on tissues alters the normal homeostasis and functioning of organs. It has been seen that inflammation-induced-lymphangiogenesis (IIL) can be inhibited with doxycycline through suppression of VEGF-C signaling. [33] It is well known that under some circumstances, macrophages can switch to a pro-angiogenic phenotype (or M2-type), which predisposes some patients to neovascular age-related macular degeneration and certain cancers. It has been shown that doxycycline can function as an adjuvant anti-angiogenic agent since it can decrease the switching of macrophages from M1 to the M2 phenotype. [34] On this case Doxycycline may prevent the late stages of HPHCoVs-associated-ARDS on which fibrosis represents the last stage. Neovascularization may also play a role as part of the alveolar hemorrhage seen in previous SARS-CoV-1 infections and that may apply to COVID-19 as well. Of note, the distortion of the lung architecture due to fibrosis may also impair the local immune response in order to clear the remaining viral particles. It may be extremely important to prevent fibrosis since it may define if the patient will develop chronic lung disease after the acute episode.

5. In a model of burn-induced microvascular hyperpermeability (typical example of acute inflammation), it was seen that the edema and extravasation was attenuated with doxycycline. [35] This could be important in the context of HPHCoVs-associated-apoptosis of epithelial and endothelial cells with vascular leakage and extravasation of fluid to the alveoli and interstitial tissue. On this case tetracyclines may provide some relief to the refractory hypoxemia and alveolar collapse seen in the worse (lethal) cases of COVID-19.
6. One of the most well-known anti-inflammatory properties of tetracyclines is the inhibition of metalloproteinases (MMP) [36]—particularly MMP-2 and 9—which has been the focus of intense research recently, applied not only to chronic gingivitis models and COPD [13, 14, 17, 18, 21, 24, 35] but to cardiovascular disease, [37] left ventricular remodeling, [38] and arterial aneurysm progression. [17] The accumulated MMP-2 and MMP-9 can aggravate pulmonary damage by degrading the basement membranes of the alveoli. [10] Not only neutrophils but also activated macrophages synthesize MMP-2 and MMP-9 de novo, and these proteinases play a role in macrophage-dependent Acute Lung injury (ALI). It has been documented that the lung of patients with SARS-CoV-1 shows massive macrophage infiltration. [6] If the histopathology of COVID-19 resembles what happens with SARS-CoV-1 and Influenza pneumonia, the inhibition of MMPs 2-9 with tetracyclines may be extremely important in controlling the final events that leads to ARDS. A recent study in H3N2-infected mice showed significantly decreased expression and activities of both MMP-2 and MMP-9 after Doxycycline treatment. [8] The above mechanism would avoid the macrophage and/or neutrophil-associated damage due to local release of not only MMPs but reactive oxygen species (ROS) as well. Paraquat (PQ) is a nonselective herbicide that has been widely used and its effect resembles what happens in viral-induced ARDS with intense neutrophilic infiltration and release of MMPs. A recent animal study showed that doxycycline could significantly inhibit the overexpression of MMP9 induced by PQ administration in both lung tissues and BALs. Doxycycline reduced neutrophil infiltration, markedly suppressed the levels of pro-inflammatory cytokines, local MPO release, and even improved the histological characteristics of the lung. [39] This is important since ARDS is the result of multiple insults (chemical or infectious) with common histopathological features that could be reversed with tetracyclines.

Possible Adverse Reactions to Tetracyclines

1. Tetracyclines have also been involved in decreasing the inflammatory response through the immunomodulatory effects on T-cells. This is the main reason of their use on T-cell mediated diseases like rheumatoid arthritis and other autoimmune diseases that are mainly T-cell mediated. [12,26] In the early 1990's, investigators had already documented these immunomodulatory effects on T-cells as a novel mechanism of action that needed to be explored. [40] It was suggested that doxycycline might decrease the inflammatory reaction in some autoimmune

diseases by eliminating activated T lymphocytes through the known process of Fas/FasL-mediated apoptosis. [41] In the case of HPHCoVs-induced-ARDS, there is already a decrease number of lymphocytes not clearly explained. Further decrease of the lymphocyte function may impair the control of viral replication and promote local viral persistence. At the same time, tetracyclines may control the cytokine storm, which may be the culprit of lymphocyte apoptosis. The net effect will need to be seen in experimental or human clinical trials.
2. Nausea, vomiting, abdominal pain, and esophagitis may be seen in patients exposed to this drug class when taken orally, which may impair the absorption of other medications in non-intubated patients.
3. Clostridium difficile colitis is also possible but less like with tetracyclines than with other antibiotics (such as cephalosporins, quinolones, and clindamycin) but some degree of dysbiosis is expectable.

Conclusions

There are only two drugs approved under EUA to treat COVID-19 patients with severe disease as discussed above. HCQ has questionable effectivity and can cause cardiac toxicity mainly when used in combination with Azithromycin. Definitively there is no evidence to use concomitant Azithromycin and is not recommended by multiple medical societies outside Clinical trials. Remdesivir showed decreased time to recovery compared with a control group without statistically significant benefit in terms of mortality. [6] New innovative, effective, and safe therapies are highly needed. Tetracyclines are well-known, inexpensive drugs that have been used for decades, and have an acceptable adverse effect profile. Tetracyclines have many anti-inflammatory properties that, theoretically, could work as an immunomodulator counteracting the exuberant inflammatory response triggered by HPHCoVs (including COVID-19). Some of the proposed mechanisms could include: decrease apoptosis of lymphocytes, endothelial, and epithelial cells; decrease the migration of neutrophils and monocytes; decrease the secretion of pro-inflammatory cytokines (including IL-6) and chemokines; decrease oxidative stress and release of ROS; inhibition of the activity of the metalloproteinases (MMP-2/9); and decrease in the progression to lung fibrosis. A direct anti-viral (either CQ, HCQ, or Remdesivir) will need to be used along with tetracyclines so it will be guaranteed the control of the viral replication and the immune response that was triggered. The combination will need to be used early in the course of the disease since during late stages (established CSS/ARDS/fibrosis) may be very difficult to reverse. Probably every patient admitted to the hospital with pneumonia has had already viral replication for some time (even while asymptomatic) and may present with early CSS, which is the optimal time to start an anti-viral (to treat remaining virions) and an immunomodulator. The intersection between decrease of viral titers and increase of cytokine release is difficult to define. This theoretical model will need to be proven in animal studies or in prospective randomized controlled clinical trials. A question that remains unanswered is if the addition of zinc to HCQ or CQ may be detrimental since it is a well-known cofactor of MMPs and Super Oxide Dismutase (SOD) both of which promotes endothelial oxidative stress.

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