

High risk COVID-19: intervening at multiple points in the COVID-19 disease process
via prophylactic treatment with azithromycin or bee derived products

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The pharmacology of azithromycin, and the actions of certain bee products, suggest the possibility of overlap with the pathophysiology of COVID-19 at several points in the disease process. First, intercellular epithelial tight junctions of the respiratory tract serve as a critical barrier to invaders. Pathophysiological factors capable of disrupting this epithelial barrier include viral virulence factors such as those observed for other coronaviruses; virulence factors derived from potentially synergistic pathogens such as *Candida albicans* and *Porphyramonas gingivalis*; and imbalances in the host inflammatory response. Azithromycin, and to a lesser extent, certain bee products, appear to have actions that oppose such processes. Second, the matrikine PGP or its derivatives may contribute to risk in individuals at high risk for serious COVID-19 infection, especially during reactivation; but azithromycin is capable of modulating PGP in some contexts. Third, the most serious COVID-19 infections are associated with massive upregulation of inflammatory cytokines such as IL-6, TNF alpha, and other inflammatory cytokines. The anti inflammatory actions of azithromycin and bee derived products such as melittin are potentially capable of modulating these processes, as well. Azithromycin is already in current use as a treatment for COVID-19; however, its utility as a protector of epithelial barrier function would be most likely to be realized in prophylactic context rather than in a treatment context. Similarly, since the anti inflammatory effects of bee products take time, their effectiveness of melittin and other bee products would be expected to be maximized in a prophylactic context. In the context of the current pandemic, prophylaxis with azithromycin, bee products, or both, might be warranted for individuals at high risk for serious COVID-19 infection.

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High risk COVID-19: potential intervention at multiple points in the COVID-19 disease process via prophylactic treatment with azithromycin or bee derived products

Abstract

The pharmacology of azithromycin, and the actions of certain bee products, suggest the possibility of overlap with the pathophysiology of COVID-19 at several points in the disease process. First, intercellular epithelial tight junctions of the respiratory tract serve as a critical barrier to invaders. Pathophysiological factors capable of disrupting this epithelial barrier include viral virulence factors such as those observed for other coronaviruses; virulence factors derived from potentially synergistic pathogens such as *Candida albicans* and *Porphyramonas gingivalis*; and imbalances in the host inflammatory response. Azithromycin, and to a lesser extent, certain bee products, appear to have actions that oppose such processes. Second, the matrikine PGP or its derivatives may contribute to risk in individuals at high risk for serious COVID-19 infection, especially during reactivation; but azithromycin is capable of modulating PGP in some contexts. Third, the most serious COVID-19 infections are associated with massive upregulation of inflammatory cytokines such as IL-6, TNF alpha, and other inflammatory cytokines. The anti-inflammatory actions of azithromycin and bee derived products such as melittin are potentially capable of modulating these processes, as well. Azithromycin is already in current use as a treatment for COVID-19; however, its utility as a protector of epithelial barrier function would be most likely to be realized in prophylactic context rather than in a treatment context. Similarly, since the anti-inflammatory effects of bee products take time, their effectiveness of melittin and other bee products would be expected to be maximized in a prophylactic context. In the context of the current pandemic, prophylaxis with azithromycin, bee products, or both, might be warranted for individuals at high risk for serious COVID-19 infection.

Author summary:

The dangerous COVID-19 pandemic has stressed medical systems around the world beyond capacity and has been a cause of tremendous loss of life. This is so even though the great majority of infections with COVID-19 are not life threatening and do not require hospitalization. Since most serious COVID-19 infections occur in people who are either of older age or suffer from chronic disease, it would seem worthwhile to focus on preventing serious illness in those people who are at greatest risk for life threatening COVID-19 infection.

Azithromycin is an antibiotic/anti-inflammatory medication that is well known to most people: it is often used for treatment of simple infections such as throat infections and bronchitis. Bee derived products such as propolis and bee venom have uses in complementary and alternative medicine — propolis is used to prevent common winter illnesses, for example, and bee venom is used in some forms of acupuncture. Since both azithromycin and bee derived products oppose multiple mechanisms by which COVID-19 could “open the door” to the respiratory tract and lungs, they would seem to be excellent choices for prevention of COVID-19 related disease in people who are at high risk.

Introduction

The search is currently underway for effective and potentially life saving treatments for the COVID-19, the new and devastating pandemic. The majority of individuals exposed to the virus do not require hospitalization; but for a minority of patients, especially those of older ages or with predisposing risk factors, the disease can be deadly. Thanks to the astonishing number of COVID-19 patients requiring hospitalization and mechanical ventilation, the current pandemic has strained healthcare systems around the world to and beyond their limits.

Azithromycin has been used with hydroxychloroquine for treatment of patients with COVID-19 infection or suspected COVID-19 infection, and there is some preliminary data that supports their use.[1] Even so, the success of such treatments may be limited when applied to patients at the peak of COVID-19 symptoms and in potential respiratory collapse.

On the basis of known pathophysiology of COVID-19 and other coronaviruses, the current discussion explores the use of two categories of agents for use in prophylaxis in individuals at high risk for serious or life threatening COVID-19 infection: the antibiotic azithromycin (and perhaps newer generation macrolides); and that of compounds derived from bee products such as bee venom and propolis,

Dysfunction of epithelial tight junctions: a pathway for viral invasion

Epithelial intercellular tight junctions define epithelial cell polarity and serve as a physical defense against pathogens. A number of factors contribute to the formation and maintenance of epithelial tight junctions, including PDZ polarity proteins and their associated complexes; zonula occludens proteins (ZO-1 and ZO-2); E-cadherin; occludin; JAM-A, and claudins 1, 3, 4, 5, and 18. It is likely that other proteins participate in this process, as well.[2,3]

Epithelial tight junctions function as a barrier capable of repelling would-be pathogenic invaders; but invasive pathogens have armaments of their own. A number of important viral pathogens encode for virulence factors capable of decreasing tight junction integrity, thus allowing for pathogenic invasion. The influenza A NS1 protein encodes for a virulence factor that binds to PDZ proteins, disrupting epithelial cell polarity and intercellular tight junctions; rhinoviruses disrupt tight junction integrity via downregulation of Zonula occludens -1 (ZO-1), a tight junction associated protein; and Human immunodeficiency virus-1 (HIV-1) effects junctional disruption both by remodeling of the cytoskeleton, by increasing claudin-2, a tight junction pore-forming protein, and by decreasing claudin-1, a tight junction sealing protein.[4-6]

Coronaviruses may target the epithelial tight junction, as well. SARS CoV-1, a human coronavirus of the respiratory tract resembling COVID-19, encodes for a virulence protein E that targets the CRUMBS3-PALS1-PATJ polarity complex. The SARS CoV-1 E protein binds to PALS1, a PDZ protein in this complex, thereby disrupting epithelial cell polarity and reducing transepithelial electrical resistance (TER).[7] Similarly, porcine epidemic diarrhea, a

gastrointestinal coronavirus in pigs, downregulates six proteins involved in maintenance of epithelial tight junctional integrity: ZO-1, ZO-2, occludin, claudin-1, claudin-4, and claudin-5.[8,9] It is not yet known whether COVID-19 or feline coronavirus participate directly in the degradation of epithelial tight junctions, but both promote secretion of TNF-alpha and IFN-gamma, cytokines that have been associated with increases in barrier dysfunction and mislocalization of tight junction proteins such as JAM-A, claudin 4, and claudin 5.[10]

By breaking down the epithelial barriers, such processes increase the potential for COVID-19 invasion and penetration.

In contrast to the above processes, azithromycin, a drug currently in use for treatment of symptomatic COVID-19, exhibits a sealing effect on respiratory tight junctions which is independent of its antimicrobial effect. Azithromycin has been found to alter processing and localization of sealant molecules such as claudin 1, claudin 4, occludin, and JAM-A; and the drug also increases TER.[11] Still, with respect to COVID-19, a question arises as to what degree this action of azithromycin could aid in ameliorating pulmonary compromise in a patient who is already exhibiting pulmonary symptoms. After all, by that point, the virus has already penetrated the respiratory epithelium. Thus, this sealing action of azithromycin on respiratory epithelial tight junctions would thus seem to show treater promise as a prophylactic. For example, it could be used to decrease severity of COVID-1 infection in high risk individuals.

Another category that might hold promise for COVID-19 prophylaxis is that of compounds derived from bees. For example, in a keratinocytes derived cell line, Brazilian Green bee propolis was found to rescue mislocalized claudin 1. Thus, compounds within bee propolis might exhibit sealant effects on epithelial tight junctions.[12] Further research is warranted.

Tight junctions and risk factors associated with severe COVID-19 infection

When it comes to the maintenance of epithelial tight junctional integrity, youth may advantage. Young mice have more tight junction-sealing claudins than do older mice. Of the junction-sealing claudins, claudins 3, 4, and 18 are the most important in the respiratory tract; and claudins 3 and 4 have been found to exhibit age-related declines in multiple tissues, including liver, pancreas, and intestine.[13] In mice, claudin-4 plays an important role in the respiratory tract: claudin-4 concentrations increase during acute lung injury and appear to enable alveolar fluid clearance.[14]

In an aging population, an additional concern is that oral and periodontal pathogens could predispose to a lessening of tight junctional integrity in areas that overlap with the respiratory tree. In the oral cavity, for example, aging leads to hyposalivation, which affects the composition of oral flora and leads to increased growth of candida species. The risk of oral candidiasis is further augmented by the presence of diabetes mellitus or dental prostheses.[15] *Candida albicans* interferes with E-cadherin, a protein shown to be essential for maintenance of intracellular tight junctions within the mucus membranes of the digestive tract[16], and is probable that the yeast does this within the oropharynx as well. By interfering with the epithelial

barrier of the oropharynx, *Candida albicans* might facilitate tissue penetration of COVID-19 or other coronaviruses from the upper respiratory tract.

Another potential challenge to tight junctional integrity of the upper airway is *Porphyromonas gingivalis*, an oral pathogen whose LPS has been found to downregulate occludin and claudin-4.[17] Interestingly, this effect was manifested by *P. Gingivalis* LPS but not by *P. Gingivalis in vitro*. The prevalence of *P. gingivalis* has been estimated to be 15% in children and approximately 50% in adults.[18,19] Dental prostheses also increase the risk for periodontitis.[20] *P. gingivalis* has been implicated as a potential pathogen in Alzheimer's disease, suggesting that the bacteria may be more pathogenic at the older end of the age spectrum.[21] Through its interference with tight junction proteins such as occludin and claudin-4, *P. gingivalis* might serve to augment the invasiveness of COVID-19 and other coronaviruses from the upper airway.

Periodontitis caused by *P. gingivalis* has been shown to respond to treatment by both azithromycin and bee venom [22-25], suggesting a prophylactic role for azithromycin, compounds derived from bee venom, or both, in suppression of *P. gingivalis* in patients at high risk for severe COVID-19 infection. Azithromycin is bactericidal for *P. gingivalis*; it reduces *P. gingivalis* biofilms in vivo, and it has been shown to reduce pro-inflammatory cytokine and chemokine production induced by *P. gingivalis* LPS. It is possible that prophylaxis with azithromycin might increase the risk for *Candida albicans*; but such side effects could be controlled via concurrent use of probiotics. Bee venom has been found to inhibit proinflammatory cytokines induced by *P. Gingivalis* LPS via suppression of NF- κ B and AP-1 signaling.

Azithromycin is already in use for treatment of COVID-19 infection. Bee venom derivatives, on the other hand, have not yet been tested in this context. However, bee venom derivatives could be of potential use in the context of COVID-19, both on account of their anti-inflammatory properties and also on account of their antimicrobial properties.[26,27] Given that paraquat-induced toxic lung injury shares a number of characteristics in common with lung injury secondary to SARS CoV1, it is of interest that melittin, an active ingredient in bee venom, has been shown to attenuate paraquat-induced lung injury in mice.[28-32] Thus, bee venom compounds might be useful in prevention of long term fibrotic sequelae, if any, due to COVID-19 infection or reactivation.

Aside from advanced age, conditions known to increase risk for severe COVID-19 infection include diabetes mellitus, smoking, and chronic lung disease. These same conditions have been found to predispose to dysfunction of tight junctions. Smokers have decreased levels of claudins 1, 3, 7, and 8; and in vitro studies have also implicated cigarette smoking in reduced expression of occludin, E-adherence, JAM-A, and ZO-1.[33] Hyperglycemia alters the expression of ZO-1 and occludin, reduces TER, and is an independent risk factor for respiratory infections.[34]

The host inflammatory immune response itself may contribute to barrier compromise. COVID-

19 infected patients have been reported to have increased IL-6, increased TNF-alpha, and increased interferon-gamma [35], all of which have been shown to impair tight junctional function in a number of epithelial cell lines. *In vitro* and in intestinal mucosa, IFN-gamma downregulates or decreases subcellular localization of ZO-1; redirects claudin, occludin, and JAM-A away from cell-cell contact regions; and effects dysfunctional changes in tight-junction associated actin structures. TNF-alpha is associated with rearrangement of epithelial tight junction-associated actin and downregulation of ZO-1 *in vitro*. [10] TNF-alpha is increased in diabetes mellitus and other hyper-inflammatory states. [36]

Thus, the elderly, diabetics, smokers, and individuals with chronic lung disease (among others) are cohorts who might stand to gain from prophylaxis during the current COVID-19 pandemic.

Proline-glycine-proline (PGP) and its connection to lung inflammation

PGP is a matrikine that has been shown to accumulate in the context of acute severe pulmonary infection, as demonstrated in animal models of Haemophilus influenzae (Hib) pneumonia and Pneumococcal pneumonia. [37] On accumulation, PGP triggers the infiltration of lung tissue by neutrophils — similar to that reported for SARS CoV1 infections.

PGP may accumulate in the context of non-infectious pulmonary disease, as well. The matrikine has been found to accumulate in the sputum of patients with chronic obstructive lung disease (COPD), with highest levels of PGP observed during exacerbations of COPD. PGP-associated exacerbations of COPD are attenuated by treatment with azithromycin. [38] PGP also appears to be detrimental to pulmonary endothelium, inducing changes that resemble ARDS. [39] The effects of azithromycin on ARDS have not been well characterized.

While PGP has not yet been demonstrated in human coronavirus infection, given the extent of neutrophil accumulation in SARS CoV1 [28], it seems reasonable to presume that PGP likely plays a role in the pathogenesis of that disease. On the other hand, patients with first time COVID-19 infections do not appear to exhibit pulmonary neutrophilia to the extent that has been reported for SARS CoV1 [40,41]. Thus, response of first time COVID-19 pulmonary infection to azithromycin is unlikely to relate to its action on PGP.

PGP may be more relevant to reactivation of COVID-19 disease: progressive neutrophilia has been observed primarily in patients with severe acute respiratory syndrome COVID-19 reactivation. [42] Thus, the observation that azithromycin reduces PGP levels suggests a potential role for azithromycin in post-recovery prophylaxis for high risk patients.

Severe COVID-19 infection and the cytokine storm

The danger of COVID-19 is believed to relate in part to cytokine storm, an exaggerated release of inflammatory cytokines potentially leading to respiratory or to multi organ system failure. Compared to COVID-patients with milder presentations, COVID-19 infected patients in the intensive care unit (those with presumed cytokine storm have been observed to manifest higher

levels of cytokines, including higher plasma levels of IL-2, IL-6, IL-7, IL-10, gCSF, IFN gamma, MAP1 alpha, and TNF alpha.[43,44]

IL6 is an inflammatory cytokine implicated in cytokine storm: it is responsible for upregulation of both the Th1 and Th2 pathways, with increased production of IL4, IL13, IFN gamma, and other inflammatory cytokines. TNF alpha upregulates IL-6, IL-6 upregulates TNF alpha, and in both COVID-19 and feline coronavirus, elevated IL6 and TNF alpha are associated with poorer prognosis.[45-47]

Azithromycin has been shown to modulate the IL-6 response in patients with severe respiratory infection and has already been suggested as a prophylactic for respiratory diseases in other contexts outside of COVID-10.[48,49] In addition to azithromycin, melittin, an anti-inflammatory compound derived from bee venom, has been shown to reduce formation of the IL6/sIL-6R complex; and whole bee venom downregulates TNF alpha and IL-6.[50-52] While melittin has not been used as a standard prophylactic agent for patients with respiratory disease, bee venom is already utilized in some forms of acupuncture for treatment of inflammatory arthritides.[53]

The anti-inflammatory cytokine IL10 downregulates inflammatory cytokines such as IL1 and TNF alpha. IL10 has been associated with improved outcomes, both in animal models of LPS associated sepsis and also in coronavirus infections.[54] In feline coronavirus, increases in IL10 play a protective role, and increases in IL10 are expected to play a protective role in the context of COVID-19, as well[47], both by attenuating cytokine storm, and also by strengthening epithelial barrier function. By reducing TNF alpha, IL10 likely exerts an (indirect) protective effect on TER and on respiratory epithelial tight junctions, thus preventing further invasion by viral particles.

Interestingly, beekeepers have been noted to have increased IL10 compared to the general population. Presumably this increase relates to chronic low level exposure to bee venom.[55] This observation suggests that, in individuals at high risk for serious COVID-19, prophylaxis with bee venom or with active ingredients contained in bee venom might be able to prevent or attenuate cytokine storm in the context of COVID-19.

Discussion

The pharmacology of azithromycin, and potentially of certain bee products, suggest the possibility of overlap with the pathophysiology of COVID-19 at several points in the disease process. Thus, azithromycin or bee derived products could have a significant role to play in the fight against the current COVID-19 pandemic for the following reasons:

First, the pathophysiology of COVID-19 most likely involves viral invasion *via* disruption of tight junctions of the respiratory epithelium — either directly as a virulence factor; *via* the host inflammatory response; *via* synergistic pathogens such as *P. gingivalis* and *C. albicans*; or *via* a combination of the above factors.

Azithromycin and bee derived compounds show promise as prophylactic agents via multiple mechanisms associated with epithelial tight junctions. Azithromycin appears to be capable of protecting epithelial tight junctions, capable of limiting the growth of potentially synergistic pathogens that harm tight junctions, and capable of modulating the inflammatory response. Bee derived products exhibit antimicrobial and anti-inflammatory actions, as well. Since these proposed interventions involve protection of barrier function, they would likely be less effective once the virus has already invaded and replicated within a host to a significant degree. Presumably these interventions would be most effective in a prophylactic context.

Second, reactivation COVID-19 infection may involve the matrikine PGP or its derivatives, especially in individuals with chronic lung disease or other risk factors. PGP, a pro-inflammatory compound that can worsen risk in individuals with COPD or respiratory associated sepsis, has been shown to be modulated by azithromycin in individuals with COPD. Thus, azithromycin holds promise as a prophylactic agent in COVID-19 post infection prophylaxis, especially in the context of COPD or other chronic lung disease.

Third, the most serious cases of COVID-19 infection appear to involve cytokine storm, a potentially deadly complication. Azithromycin and bee products modulate the inflammatory response to a moderate degree, but such modulation is likely inadequate in the context of full-blown cytokine storm. Thus, these anti-inflammatory actions, too, would appear to be most effective in a prophylactic context.

Since azithromycin is currently utilized for long term prophylaxis in other contexts, its adoption during the COVID-19 pandemic should be relatively easy to implement. Even though this strategy carries the risk of increased antibiotic resistance, the pay-off might be worthwhile in the context of the current pandemic.

Bee derived products are a less familiar option, but one advantage of these products is that they do not carry the risk of increased antibiotic resistance. Further research is needed to isolate effective active ingredients from these products while limiting potential complications.

Given all of the above potential loci for intervention, it is proposed that azithromycin, bee derived products, or a combination of these be considered for prophylaxis in individuals at high risk for serious COVID-19 infection.

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