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Review Article

Repurposing Donepezil to Treat COVID-19: A Call for Retrospective Analysis of Existing Patient Datasets

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Abstract

We hypothesize that patients treated with donepezil will show decreased risk of death following COVID-19 infection. The acetylcholinesterase antagonist donepezil is widely prescribed for early-stage Alzheimer's disease. Clinical studies show donepezil treatment decreases risk of death from pneumonia and myocardial infarct, improves vascular function and inhibits risk of stroke. Preclinical studies indicate donepezil itself or increased acetylcholine more generally protect from fatal Acute Respiratory Distress Syndrome (ARDS), sepsis, cytokine storm, cardiovascular damage, Disseminated Intravascular Coagulation (DIC) and acute kidney damage. Donepezil is also an agonist of the anti-viral sigma-1 receptor. In the United States, there have been over 600,000 confirmed cases of COVID-19 infection and over 170,000 deaths associated with nursing home outbreaks. Rather than run a new clinical trial, our hypothesis can be tested through retrospective analysis of data obtained from nursing home or hospital databases collected at the county, state, or federal level. We believe retrospective analysis of this data will show donepezil treatment shortens hospital stay decreases overall risk of hospitalization and death. The data could be rapidly translated into clinical practice to prevent COVID-19 fatalities in the most vulnerable patient populations.

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Keywords

Cholinergic Anti-Inflammatory Pathway; Donepezil; Acetylcholine; Acetylcholinesterase; $\alpha 7$ Nicotinic Acetylcholine Receptor; $\alpha 7$ nachr; Inflammaging; COVID-19; Racial Disparity; Sigma-1 Receptor; Immunity; Acute Respiratory Distress Syndrome; Sepsis; Vascular Damage; Heart Failure; Disseminated Intravascular Coagulation

Abbreviations

CAP: Cholinergic Anti-Inflammatory Pathway; AD: Alzheimer's Disease; ARDS: Acute Respiratory Distress; DIC: Disseminated Intravascular Coagulation

Introduction

The elderly (particularly elderly men) and members of racial minorities are at the highest risk of death following SARS-CoV-2 infection [1-12]. These same groups exhibit increased systemic inflammation and decreased overall immunity, associated with increased morbidity and greater risk of death following respiratory viral infection [13-23]. Systemic inflammation also increases risk of cardiovascular disease, stroke and sepsis [24-31]. The results of these future studies could identify more targeted immunomodulatory strategies beyond steroid administration

Since these are all features of fatal COVID-19 infection, decreasing systemic inflammation represents a potential therapeutic strategy to minimize SARS-CoV-2 morbidity and mortality in these high-risk populations.

Systemic inflammation is regulated through the efferent arm of the brain-immune system circuit known as the Cholinergic Anti-Inflammatory Pathway (CAP), which induces release of the key anti-inflammatory mediator ACh at sites of inflammation [32-34]. ACh binding to $\alpha 7$ nicotinic ACh receptors on inflammatory macrophages decreases activation/nuclear translocation of the NF- κ B transcription factor, thereby reducing inflammatory cytokine production [35-39]. ACh also acts on muscarinic receptors present on multiple cell populations to alter inflammation and limit neutrophil influx.[40-43]. There is an inverse relationship between cholinergic function (i.e., production of and response to ACh) and systemic inflammation, even in otherwise healthy individuals [24]. ACh and cholinergic function decrease as age-related systemic inflammation (inflammaging) and associated age-related disorders increase [44]. We propose that increasing ACh availability will reverse systemic inflammation and greatly decrease the overall risk of lung damage, heart failure, kidney damage, vascular damage and death in high-risk persons infected with the novel SARS-CoV-

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2 virus. This can be accomplished by repurposing the Aetylcholinesterase (AChE) antagonist donepezil.

Donepezil increases systemic ACh availability by blocking AChE-mediated hydrolysis. It has been used to treat early-stage Alzheimer's Disease (AD) for over 20 years [45]. Research into donepezil has largely focused on neurological symptoms and disease progression; however, patient studies and retrospective analyses have shown that patients on donepezil therapy exhibit decreased inflammation and improved respiratory and cardiovascular function [46]. Respiratory and cardiovascular failure are leading causes of death in the elderly and characteristic features of fatal COVID-19 infection [47,48]. The most common clinical finding in fatal COVID-19 is respiratory failure, including pneumonia and Acute Respiratory Distress (ARDS) resulting from an overactive inflammatory immune response. Additional clinical findings often noted in fatal disease include myocardial damage, acute kidney injury and Disseminated Intravascular Coagulation (DIC) [1-4]. Human retrospective cohort analyses and pre-clinical studies have shown therapeutic efficacy of donepezil itself or increased ACh more generally to limit morbidity and prevent death from each of these causes.

Respiratory Protection

Even before the SARS-COV2 pandemic, pneumonia was a leading cause of death in the elderly [47,48]. Retrospective analyses from over 25,000 patients hospitalized for pneumonia showed that mortality rates were 64% lower in AD patients treated with donepezil compared to untreated AD patients. A similar degree of protection (63%) was noted when the donepezil treated AD patients were compared to age-matched patients who did not have dementia, indicating that protection was not due solely to reversing AD-specific risk factors [49].

The correlation between increased ACh and decreased risk of death from respiratory infection is consistent with a large body of preclinical evidence illuminating the critical role of cholinergic signal transmission in maintaining normal lung structure and function as well as inhibiting immune-mediated inflammation leading to ARDS [50-58]. Key to respiratory protection is ACh receptor ligation-induced changes in pulmonary macrophage gene expression [59-64]. Pulmonary macrophages initiate inflammation and regulate local immunity at the outset of infection, then orchestrate immune resolution and tissue repair at its conclusion [65]. This diverse cellular function is due to macrophage gene expression plasticity known as polarization. All macrophages exist on a polarization continuum, with extremes canonically defined as inflammatory M1 macrophages vs anti-inflammatory/tissue repair M2 macrophages. M1 macrophages produce high levels of proinflammatory cytokines and are critical to mounting an effective anti-pathogen innate immune response. Conversely, M2 macrophages produce anti-inflammatory cytokines to resolve ongoing inflammation while initiating tissue repair [66]. In the lungs, this polarization shift from inflammatory M1 to reparative M2 is key

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to resolving pulmonary inflammation and inducing tissue repair after infection [67]. Combined preclinical and clinical evidence indicates increased ACh/cholinergic signal transmission alters inflammatory cytokine expression, limits pulmonary damage and decreases overall morbidity and mortality during respiratory injury and infection. Decreasing ACh availability increases inflammatory cytokine production, resulting in extended pulmonary damage and increased morbidity and mortality [59-64].

The lungs contain multiple sources of ACh including the pulmonary vagal nerve, the airway epithelium and cholinergic lymphocytes [55,68,69]. ACh acts as an autocrine/paracrine hormone for the airway epithelium, regulating differentiation of the critical airway epithelial basal cells and proliferation of the airway epithelium while protecting against cytokine-induced barrier dysfunction and limiting neutrophil influx into the airways [43,70-72]. The number of lung cholinergic lymphocytes greatly increases during respiratory viral infection during generation of the cellular immune response. These cholinergic lymphocytes are found in direct physical contact with inflammatory macrophages during the transition period from peak cellular immunity to immune resolution and tissue repair, indicating a mechanism for ACh delivery to specific cellular targets [73]. Decreasing cholinergic signal transmission during respiratory viral infection inhibits pulmonary M2 macrophage polarization, leading to extensive pulmonary inflammation, abnormal tissue remodelling, increased morbidity and delayed recovery [73,74]. Together, the preclinical and clinical evidence indicates that increased ACh/cholinergic signal transmission limits pulmonary damage and decreases overall morbidity and mortality during respiratory infection, supporting the therapeutic use of donepezil to mitigate illness associated with COVID-19 infection.

Myocardial Protection

Acetylcholine plays a critical role in normal cardiac function [75]. A retrospective analysis of the Swedish National Patient Registry and the Swedish Dementia Registry (which covers ~90% of all Swedish patients with a dementia diagnosis) examined risk of Myocardial Infarct (MI) and death in dementia patients treated with AChE inhibitors (including donepezil as well as rivastigmine and galantamine). With a sample size of over 7,000 patients, they found that AChE inhibitor use was associated with a 35% decreased risk of MI or death. Reduced risk associated with AChE treatment was consistent when groups were matched by age, sex, type of dementia and presence or absence of established coronary vascular disease. The authors comment that therapeutic use of AChE inhibitors could improve cardiac function, reduce supplemental oxygen use and reduce risk of MI and death [76]. These findings were extended in a recent meta-analysis of 9 cohort studies demonstrated a 37% reduction in cardiovascular events including stroke, myocardial infarction, acute coronary syndrome and cardiovascular mortality in patients treated with AChE inhibitors [77]. Preclinical studies further illustrate the protective function of ACh in models of myocardial infarction/ischemia-reperfusion, viral

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myocarditis and endotoxin-induced myocardial damage [31]. Cholinergic manipulation is being explored as a strategy to minimize myocardial inflammation, given the strong correlation between inflammation/inflammaging and cardiovascular disease development [28,30,31].

Vascular Protection

Disseminated Intravascular Coagulation (DIC) is noted in over 50% of fatal COVID cases [2, 3]. During viral infection, Tissue Factor (TF) regulates activation of the coagulation system. Vascular endothelial cells release TF into the circulation in response to vascular damage or inflammatory cytokines, a process that is a critical step in DIC development and is increased in COVID 19 [78,79]. Donepezil could limit development of DIC through several mechanisms. First, donepezil therapy is associated with overall vascular protection. ACh acts directly on the vascular endothelium to induce vasodilation [80]. Human studies indicate donepezil-treatment improves vasodilatory responses and AD patients show decreased evidence of vascular damage after donepezil therapy [81,82]. Second, donepezil therapy reduces inflammatory cytokine production. In AD patients, donepezil therapy decreases serum concentrations of TNF, IFN γ , IL1 β and IL6 [83-87]. Reduced exposure to inflammatory cytokines and improved vascular structure would limit endothelial TF release. Third, ACh directly inhibits platelet activation [88]. Diminished platelet activation would limit the overall blood clotting, further reducing inflammatory cytokine induction, local vascular damage and TF release. Together, these would act to decrease risk of myocardial and cerebral infarcts and DIC.

Kidney Protection

Acute Kidney Injury (AKI) is present in over 40% of critically ill patients with SARS-CoV-2 infection [1-3]. In preclinical models of acute kidney failure, increased ACh is associated with protection from induced renal dysfunction [89]. Donepezil treatment reduced tubular damages, prevented neutrophil infiltration and decreased inflammatory cytokines, nitric oxide content and oxidative damage [90]. Acute kidney injury due to ischemia-reperfusion and sepsis is also prevented through CAP induction via vagal nerve stimulation, further indicating a role for increased ACh in renal protection [91-93].

Anti-Viral Activity

Donepezil has additional potential as an anti-viral therapeutic beyond improving inflammatory regulation. Peripheral blood lymphocytes from donepezil-treated AD patients are resistant to in vitro infection with vesicular stomatitis virus [94]. Mathematical modeling showed only a

34% correlation of between resistance to infection and altered NF- κ B activation. The authors hypothesized the existence of additional, undefined protective mechanism(s) associated with donepezil treatment [95]. One potential protective mechanism involves the sigma-1 receptor. Donepezil acts as a high-affinity agonist for sigma-1 receptors [96]. Sigma-1 receptor agonists may directly inhibit early stages of viral infection and replication of SARS-CoV-2 [97]. The sigma-1 agonist fluvoxamine has shown efficacy in decreasing clinical progression and reducing risk of hospitalization [98,99]. This has led to a search for additional sigma 1 receptor agonists as potential treatment for SARS-CoV-2 infection, further supporting exploration of donepezil as a COVID-19 therapeutic [100].

Donepezil: Proposed Retrospective Analysis

The preceding section outlined our reasoning supporting donepezil as a potential therapy for those in high-risk groups associated with increased inflammation. Previous reports have also discussed manipulating cholinergic signal transmission as a COVID-19 therapeutic strategy [73,101-105]. There are four clinical trials currently underway examining manipulation of the CAP to treat COVID-19. Three trials are examining the ability of nicotine patches and one is testing the AChE antagonist pyridostigmine to prevent infection and decrease morbidity and mortality in infected patients [106,107]. Rather than initiating an additional trial, we propose retrospective analysis of existing data sets to examine the correlation between donepezil therapy and risk of hospitalization or death from COVID-19 infection.

Donepezil is a widely prescribed drug. There are over 5 million prescriptions written for donepezil every year in the United States. Since 95% of AD patients are age 65 and older, the dosage and safety profiles for the elderly have been well studied [46]. Almost one-half (46%) people in federally licensed residential care communities or nursing homes have dementia [108-111]. Unfortunately, these facilities have been the site of numerous COVID-19 outbreaks. As of 2/7/21, there have been over 600,000 confirmed cases of COVID-19 and over 127,000 deaths among residents of 15,333 nursing homes/residential care communities in the United States alone [112]. Similar outbreaks have taken place in residential care facilities around the world [113]. Data from these outbreaks represent the equivalent of an extended, multinational clinical trial examining the impact of donepezil prophylaxis in COVID-19 infection.

Using a retrospective cohort study design, de-identified patient data from confirmed COVID-19 infections could be separated by AD status and use of the AChE antagonist donepezil, leading to three groups: (a) donepezil-treated AD patient; (b) AD patients not treated with donepezil (or any AChE inhibitor); and (c) non-AD patient. The primary endpoint would be risk of death (from any cause) within one month of COVID diagnosis. Secondary endpoints could include need for supplemental oxygen, overall risk of hospitalization and length of hospital stay. Results would be stratified by age (65-74, 75-84, > 85), sex and race. In the

United States, one such data source would be Medicare claims [114]. Similar data sources exist for other countries as well, so results can be confirmed multilaterally [49,76]. Since protection would be due to physiological responses rather than direct anti-viral activity, any therapeutic efficacy should be resistant to viral evolution.

Conclusion

The literature supports repurposing of donepezil to prevent severe COVID-19 in the populations at the greatest risk. Future studies could explore the impact of donepezil on risk of/recovery from “long COVID” or treatment efficacy in younger populations. However, even if our hypothesis regarding donepezil is incorrect, the strategy of retrospective data analysis to search for therapeutic strategies should be far more wide-spread than is currently the case. This is particularly true for the ongoing pandemic. The combined outbreaks in nursing home, assisted living and memory care facilities constitute a massive, unplanned clinical trial. All that remains is to analyze the data.

Conflicts of Interest

The authors declare that have no competing interest and not any conflict of interest.

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