COVID-19-related myocarditis and cholinergic anti-inflammatory pathways

Weike Liu, Zhendong Liu, Yue-Chun Li

PII: S1109-9666(20)30286-4
DOI: https://doi.org/10.1016/j.hjc.2020.12.004
Reference: HJC 587

To appear in: Hellenic Journal of Cardiology

Received Date: 29 September 2020
Revised Date: 19 November 2020
Accepted Date: 3 December 2020


This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Hellenic Society of Cardiology. Publishing services by Elsevier B.V. All rights reserved.
Activation of efferent vagus nerve

Macrophages or T-cells

Inhibition of pro-inflammatory cytokines expression

IL-1β, IL-6, TNF-α, and other pro-inflammatory cytokines

Cardiomyocyte

Inhibition of ACE2 expression

ACE2

SARS-CoV-2

Cytokine storm Hyperinflammation

Direct injury

Myocarditis
COVID-19-related myocarditis and cholinergic anti-inflammatory pathways

Short title: Myocarditis and cholinergic anti-inflammatory pathways

Weike Liu1, Zhendong Liu2,*, Yue-Chun Li1,*

1 Department of Cardiology, Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, 325000, China
2 Basic Medicine College, Shandong First Medical University, Jinan, Shandong, 250062, China

*Corresponding author: Yue-Chun Li, Ph.D.
Department of Cardiology, Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University,
No. 109, Xueyuan Road, Wenzhou, Zhejiang, 325000, China.
Tel.: 86-0577-88002216
Fax: 86-0577-88832693
E-mail: liyuechun1980@sina.com

*Corresponding author: Zhendong Liu, M.D.
Basic Medicine College, Shandong First Medical University,
No. 18877, Jingshi Road, Jinan, Shandong, 250062, China.
Tel.: 86-0531-82919716
Fax: 86-0531-82919937
E-mail: zhendongliu876@126.com

Word count: 1,992
Number of References: 86
Abstract

Coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is currently in a pandemic outbreak and has become a global health issue. In addition to primarily involving the respiratory system, myocarditis is considered an important and fatal lesion in patients with COVID-19. However, effective therapeutic methods are currently lacking. The cholinergic anti-inflammatory pathway (CAP) has been demonstrated to suppress pro-inflammatory cytokine production and control inflammation in sepsis and other medical conditions. Therefore, the CAP may be a potential and effective therapeutic method for COVID-19-related myocarditis. This article reviews the relationship between COVID-19-related myocarditis and the CAP and discusses the CAP as a potential therapeutic modality in the treatment of COVID-19-related myocarditis.

Key Words: Severe acute respiratory syndrome coronavirus-2; Myocarditis; Cholinergic anti-inflammatory pathway
1. Introduction

The coronavirus disease COVID-19, whose current outbreak has resulted in a pandemic with significant mortality,\textsuperscript{1-5} is caused by a novel single-stranded RNA virus between 26 and 32 kb in length; this virus is the seventh known corona virus to infect humans and was named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the WHO.\textsuperscript{6,7} Similarly to the Severe Acute Respiratory Syndrome (SARS) outbreak in 2002 and Middle East Respiratory Syndrome (MERS) outbreak in 2012,\textsuperscript{7-10} COVID-19 quickly spread worldwide and has affected human health and the economy on an unprecedented scale since its initial outbreak in December 2019.\textsuperscript{2-4,11} Although the case fatality rate is less than that of SARS (9.6%) and MERS (34.4%), COVID-19 has claimed more lives than SARS and MERS combined.\textsuperscript{3,7,12}

The primary organs involved in COVID-19 are those in the respiratory system, which is affected by acute respiratory distress syndrome (ARDS).\textsuperscript{6,11} However, multiorgan damage/failure develops in most cases.\textsuperscript{6,13-17} The myocardium and immune system appear to be particularly susceptible to SARS-CoV-2.\textsuperscript{6,14-19} Myocarditis, a specific cardiovascular manifestation with fatal outcomes, has been reported to be a potential etiology underlying myocardial injury in patients with COVID-19.\textsuperscript{12,20-24} A retrospective multicenter study has attributed 40% (29 patients) of cases to myocarditis with circulatory failure or respiratory failure among 68 fatal cases of COVID-19.\textsuperscript{25} A “cytokine storm” triggered by immunological dysregulation is considered to underlie COVID-19-related myocarditis,\textsuperscript{6,26} although the mechanisms of myocardial inflammation in COVID-19 remain unclear.\textsuperscript{27,28}

The cholinergic anti-inflammatory pathway (CAP), a neuro-immunomodulatory pathway, suppresses pro-inflammatory cytokine production and controls inflammation in sepsis and other medical conditions.\textsuperscript{29-34} For therapeutic purposes, CAP activation can ameliorate lung injury,\textsuperscript{15} rheumatoid arthritis,\textsuperscript{35,36} acute kidney injury,\textsuperscript{37} and Alzheimer’s disease.\textsuperscript{38-41} Recently, the CAP has been recommended for patients with COVID-19.\textsuperscript{34,42}

In this article, we briefly review COVID-19-related myocarditis and the CAP, and we discuss the CAP as a therapeutic modality in the treatment of COVID-19-related myocarditis.

2. Incidence of COVID-19-related myocarditis

Biopsy studies have indicated that the incidence of viral etiology ranges between 37.8% and 77.4% among patients with acute myocarditis in Europe.\textsuperscript{6,43,44} Although the true prevalence of COVID-19-related myocarditis is unknown,\textsuperscript{7} acute myocardial injury/damage appears to be common in
patients with COVID-19. Clinical observations suggest that approximately one-quarter of hospitalized patients with COVID-19 have acute myocardial injury/damage with elevated cardiac tropon in levels and as much as 7% of deaths are attributable to COVID-19-related myocarditis. A meta-analysis including 26 studies and 11685 patients with COVID-19 infection has reported a weighted pooled prevalence of acute myocardial injury of 20%. The prevalence ranged from 5% to 38%, depending on the diagnostic criteria used in the studies. Recently, Belot and coworkers have reported that the rate of myocarditis is as high as 70% in COVID-19 cases with pediatric inflammatory multisystem syndrome.

Early case reports on COVID-19-related myocarditis were sporadic. Ruan and colleagues first reported that myocarditis may be caused by SARS-CoV-2 infection. With the development of the COVID-19 pandemic, reports of COVID-19-related myocarditis cases have increased. In a retrospective multicenter study conducted to investigate the causes of death in patients with COVID-19 by using the database of the Jin Yin-tan Hospital and Tongji Hospital in China, among 68 deaths due to COVID-19 infection, 7% (five patients) died of myocarditis with circulatory failure, and 33% (22 patients) died of myocarditis and respiratory failure. These results indicate that myocarditis may contribute to the death of patients with COVID-19.

The first direct evidence of COVID-19-related myocarditis by endomyocardial biopsy (EMB, regarded as the gold standard for diagnosis of myocarditis) was reported in a 43-year-old woman with COVID-19. Diffuse T-lymphocytic inflammatory infiltrates with apparent interstitial edema and limited focal necrosis were documented, and no replacement fibrosis was detected with EMB. However, the SARS-CoV-2 genome was not detected within the myocardium. COVID-19-related fulminant myocarditis has even been diagnosed in a 2-year-old infant. Autopsy studies have revealed detection of SARS-CoV-2 mRNA in the myocardium in five out of 12 COVID-19 victims.

Beyond acute and life-threatening myocarditis in active COVID-19 infection, myocardial inflammation may also evolve as a delayed sequela of healed COVID-19. For example, Wenzel and colleagues have detected the expression of SARS-CoV-2 specific nucleic acid by EMB in two patients who were negative for COVID-19 according to nasopharyngeal swab testing; the authors found a positive result for the SARS-CoV-2 genome. A 31-year-old male diagnosed with myocarditis after COVID-19 recovery and discharged after 3 weeks has been speculated to have residual myocardial inflammation as a result of COVID-19.
3. The mechanism underlying COVID-19-related myocarditis

The mechanism underlying COVID-19-related myocarditis is not well understood. The most plausible mechanisms are downregulation of angiotensin-converting enzyme (ACE)2 expression and a hyper-inflammatory cytokine storm leading to myocarditis.\textsuperscript{51} ACE2 is highly expressed and attached to the cell membranes in the lung, heart, intestine, blood vessels, and other tissues; it is the host target receptor mediating SARS-CoV-2 cell entry.\textsuperscript{12,18,51} Compared with those of other SARS-CoVs, the structure of the SARS-CoV-2 binding site is more compact, with greater binding stability and significantly enhanced binding affinity to the ACE2 receptor.\textsuperscript{11,61}

ACE2 has demonstrated immunoreactivity in cardiac myocytes.\textsuperscript{62-65} SARS-CoV-2 infection considerably downregulates ACE2 expression and impairs ACE2 function.\textsuperscript{66-68} The disruption of ACE2 impedes the effects of the protective signaling pathways in cardiac myocytes via increasing the release of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-6.\textsuperscript{66-68}

Another probable mechanism is that SARS-CoV-2 infection may trigger a local immune response, recruit monocytes and T-cells, and release cytokines and chemokines, thus resulting in an inflammatory cytokine storm.\textsuperscript{6,17} Macrophage and T-lymphocytic cells have been confirmed to play a critical role in COVID-19-related myocarditis.\textsuperscript{7,57,60} In a 69-year-old patient from Italy with COVID-19-related myocarditis, large, vacuolated, and CD68\textsuperscript{+} macrophages with membrane damage and cytoplasmic vacuoles have been observed to infiltrate into the myocardium, on the basis of EMB with immunological light microscopy.\textsuperscript{59} Meanwhile, SARS-CoV-2 particles were detected in cardiac macrophages but not cardiomyocytes.\textsuperscript{59} A 43-year-old patient diagnosed with COVID-19-related myocarditis has been documented to have diffuse T-lymphocytic inflammatory infiltrates with myocardial edema, despite SARS-CoV-2 negativity, by using EMB.\textsuperscript{57} These data indicate that macrophage and T-lymphocytic cells play critical roles in COVID-19-related myocarditis.

Pathological monocytes and T-cells mediate the hyper-inflammatory cytokine storm in viral infection.\textsuperscript{51} SARS-CoV-2 infection induces the cytokine storm, mediated through monocytes and T-cells, thus leading to myocarditis.\textsuperscript{51} The cytokine storm caused by SARS-CoV-2 infection indicates an immune system gone awry and manifests as excessive increased plasma concentrations of pro-inflammatory cytokines such as IL-1β, IL-2, IL-6, IL-10, granulocyte colony-stimulating factor, interferon-γ-inducible protein 10, macrophage inflammatory protein 1 alpha, monocyte chemoattractant protein 1, and TNF-α.\textsuperscript{27} The pro-inflammatory cytokines spread throughout the body, including the
heart, via the systemic circulation, even as the cytokine storm occurs in local organs or tissues. Thus, dysregulated immune and inflammatory function is important in COVID-19-related myocarditis.

4. The role of the cholinergic anti-inflammatory pathway (CAP) in COVID-19-related myocarditis

In the past few decades, understanding of the critical role of the neuroimmune system has been enhanced by clarification of the overlapping distributions and interaction between the nervous system and immune system in the regulation of immunological and inflammatory responses. Among the neuroimmune interactions, the vagus nerve generated considerable interest when it was characterized as a major regulator of inflammation. Borovikova and colleagues have demonstrated that acetylcholine (ACh), the principle vagal neurotransmitter, significantly attenuates inflammation in the human macrophage response to lipopolysaccharide (LPS). An interesting and important finding is that the level of circulating TNF-α is significantly increased by bilateral cervical vagotomy in rats administered LPS but is markedly decreased when the distal end of the efferent vagus nerve is stimulated by constant voltage pulses in vagotomized rats administered LPS. Therefore, the vagus nerve pathway, termed the CAP, plays a major role in the neural control of inflammation and the neuroimmune dialogue.

We hypothesized that the CAP, coinciding with the mechanism underlying COVID-19-related myocarditis, may play a crucial role in inhibiting inflammation in COVID-19-related myocarditis directly and/or via ACE-2. The principle vagal neurotransmitter ACh should bind acetylcholine receptors, particularly α7 nicotinic ACh receptors (α7nAChR), and consequently control immune cells and inhibit the production of inflammatory cytokines. The α7nAChR is composed of five identical α7 subunits and is the central component of the CAP influencing anti-inflammatory cells. It is encoded by AHRNA7on chromosome 15q14 and is widely expressed on the surfaces of inflammatory cells including macrophages, monocytes, T cells, B cells, and dendritic cells. Wang and colleagues have found that the serum levels of TNF-α, interleukin (IL)-1β, and IL-6 are significantly higher in endotoxemic α7nAChR−/− mice than wild-type mice. When electrical stimulation is applied to the vagus nerve, endotoxin-induced serum TNF levels are significantly attenuated in wild-type mice but not in α7nAChR−/− mice. Experiments in human macrophages and mouse peritoneal macrophages have further demonstrated that the release of TNF-α induced by LPS from macrophages is inhibited by Ach and nicotine through stimulation of α7nAChR. In monocytes,
α7nAChR activated by non- or strong agonists downregulates NF-κB nuclear translocation and TLR4 expression, and suppresses transcription of pro-inflammatory cytokines. De-Pu and colleagues have used nicotine to activate α7nAChR in mice with acute virus-induced myocarditis. The proportion of Th2 and Treg cells increased, and that of Th1 and Th17 cells decreased in the spleen, thus indicating that the α7 subunit is essential for cholinergic suppression of TNF-α and the normal regulation of systemic inflammatory responses.

Recently, several studies have demonstrated that α7nAChR is an important mediator of nicotine’s upregulation of ACE-2, the main receptor used by SARS-CoV-2 to enter host cells. The repurposing of α7nAChR antagonists has been proposed as a method to alter ACE-2 expression and prevent SARS-CoV-2 entry, although the interaction between SARS-CoV-2 and CAP requires further investigation. Beyond providing an entry point for SARS-CoV-2 into cardiac myocytes and suppressing inflammatory cytokines, ACE-2 interaction with SARS-CoV-2 facilitates detrimental effects on parasympathetic tone and cardiovascular regulation. Therefore, targeting the CAP, particularly α7nAChR, through vagus nerve stimulation (VNS) might be a useful therapeutic measure for patients with COVID-19-related myocarditis.

5. CAP as a prospective therapy for COVID-19-related myocarditis

Extending the seminal finding that the CAP inhibits acute inflammation, researchers have assessed the therapeutic potential of VNS and have reported promising therapeutic results for many diseases including sepsis, dementia, arthritis, and cerebro- and cardio-vascular diseases. Koopman has found that the serum levels of TNF-α, IL-1β, and IL-6 significantly decrease, and the clinical symptoms improve, in patients with rheumatoid arthritis after 3 months of treatment with an implantable VNS device. In patients undergoing off-pump surgical revascularization, the peripheral blood production of TNF-α and IL-6 significantly decreases after 6 hours of VNS treatment. Additionally, some clinical trials aiming to assess the effects of VNS in diseases such as heart failure, atrial fibrillation, and traumatic brain injury are currently in progress.

Because of its safety and the absence of significant adverse effects, VNS has also been proposed as a potential therapeutic strategy for the current outbreak of COVID-19. Staats and coworkers have reported that patients with COVID-19 show expedited symptomatic recovery from severe cough, chest tightness, and shortness of breath after non-invasive VNS. Leung and Russo have suggested that methyllycaconitine or α-conotoxin, repurposed α7nAChR antagonists, are potential medications for
COVID-19, although this usage should be approached with caution.\textsuperscript{79}

Importantly, the CAP has been shown to decrease the inflammatory response in viral myocarditis.\textsuperscript{76,86} Activated $\alpha 7nAChR$ by nicotine improves the balance of Th1/Th2 and Th17/Treg cell functional axis\textsuperscript{76} and significantly down-regulates the expression of TNF-$\alpha$ and IL-6 in mice with CVB3-induced viral myocarditis.\textsuperscript{86} In addition, $\alpha 7nAChR$ plays an important role in the expression of ACE-2, the target receptor of SARS-CoV-2.\textsuperscript{66,77,78} Thus, CAP might be a promising and effective therapeutic avenue for treating COVID-19-related myocarditis.

6. Conclusions and expectations

In summary, the CAP plays an important role in COVID-19-related myocarditis. Targeted activation of the CAP might provide a potential and effective therapeutic method for SARS-CoV-2 induced myocarditis in the COVID-19 pandemic. However, the interaction between the CAP and SARS-CoV-2 infection, including COVID-19-related myocarditis, must be further investigated. Moreover, large sample, multicenter, and multi-ancestry prospective clinical trials are needed to further extensively assess the anti-inflammatory effects of the CAP in COVID-19-related myocarditis.

Funding

This work was supported by the National Natural Science Foundation of China (grant Nos. 81870281 and 81670432), the Zhejiang Provincial Natural Science Foundation of China (grants Nos. LY18H020011 and LQ19H020005), and the Academic Promotion Program of Shandong First Medical University.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Competing interests

The authors have no financial conflicts of interest.

References


2020;323(20):2052-2059.


[66] Fudim M, Qadri YJ, Ghadimi K, MacLeod DB, Molinger J, Piccini JP, Whittle J, Wischmeyer PE,


Legends:

Graphic Abstract: The cholinergic anti-inflammatory pathway might be a promising and effective therapeutic method for COVID-19-related myocarditis. ACh indicates acetylcholine; α7nAChR, α7 nicotinic acetylcholine receptors; ACE2, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; IL, interleukin; TNF-α, tumor necrosis factor-α.