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## Review Article

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

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## ABSTRACT

Coronavirus disease-2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2, is currently in a pandemic outbreak and has become a global health issue. In addition to the primarily involvement of 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.

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## 1. Introduction

The coronavirus disease-2019 (COVID-19), whose current outbreak has resulted in a pandemic with significant mortality,<sup>1–5</sup> 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.<sup>6,7</sup> Similar to the Severe Acute Respiratory Syndrome (SARS) outbreak in 2002 and Middle East Respiratory Syndrome (MERS) outbreak in 2012,<sup>7–10</sup> COVID-19 quickly spread worldwide and has affected human health and the economy on an unprecedented scale since its initial outbreak in December 2019.<sup>2–4,11</sup> 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.<sup>3,7,12</sup>

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

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

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.<sup>6,43,44</sup> Although the true prevalence of COVID-19-related myocarditis is unknown,<sup>7</sup> acute myocardial injury/damage appears to be common in patients with COVID-19.<sup>10,45</sup> Clinical observations suggest that approximately one-quarter of hospitalized patients with COVID-19 have acute myocardial injury/damage with elevated cardiac troponin in levels<sup>7,46–49</sup> and as much as 7% of deaths are attributable to COVID-19-related myocarditis.<sup>12,50</sup> 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%.<sup>51</sup> 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.<sup>52</sup>

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.<sup>25</sup> With the development of the COVID-19 pandemic, reports of COVID-19-related myocarditis cases have increased.<sup>10,25,53–56</sup> 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,<sup>25</sup> 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.<sup>25</sup> These results indicate that myocarditis may contribute to the death of patients with COVID-19.<sup>12</sup>

The first direct evidence of COVID-19-related myocarditis by endomyocardial biopsy (EMB, regarded as the gold standard for the diagnosis of myocarditis) was reported in a 43-year-old woman with COVID-19.<sup>57</sup> 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.<sup>53</sup> Autopsy studies have revealed the detection of SARS-CoV-2 mRNA in the myocardium in five out of 12 COVID-19 victims.<sup>58</sup>

Beyond acute and life-threatening myocarditis in active COVID-19 infection, myocardial inflammation may also evolve as a delayed sequela of healed COVID-19.<sup>6,55,56,59,60</sup> 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.<sup>56</sup> 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.<sup>55</sup>

## 3. The mechanism underlying COVID-19-related myocarditis

The mechanism underlying COVID-19-related myocarditis is not well understood. The most plausible mechanisms are the downregulation of angiotensin-converting enzyme (ACE)2 expression and a hyper-inflammatory cytokine storm, which lead to myocarditis.<sup>51</sup> 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 that mediates SARS-CoV-2 cell entry.<sup>12,18,51</sup> 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.<sup>11,61</sup>

ACE2 has demonstrated immunoreactivity in cardiac myocytes.<sup>62–65</sup> SARS-CoV-2 infection considerably downregulates ACE2 expression and impairs ACE2 function.<sup>66–68</sup> The disruption of ACE2 impedes the effects of the protective signaling pathways in cardiac myocytes by increasing the release of pro-inflammatory cytokines, including tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-6.<sup>66–68</sup>

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.<sup>6,17</sup> Macrophage and T-lymphocytic cells have been confirmed to play a critical role in COVID-19-related myocarditis.<sup>7,57,60</sup> In a 69-year-old patient from Italy with COVID-19-related myocarditis, large, vacuolated, and CD68<sup>+</sup> macrophages with membrane damage and cytoplasmic vacuoles have been observed to infiltrate into the myocardium, on the basis of EMB with immunological light microscopy.<sup>59</sup> Meanwhile, SARS-CoV-2 particles were detected in cardiac macrophages but not cardiomyocytes.<sup>59</sup> 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.<sup>57</sup> 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.<sup>51</sup> SARS-CoV-2 infection induces the cytokine storm, mediated through monocytes and T-cells, thus leading to myocarditis.<sup>51</sup> The cytokine storm caused by SARS-CoV-2 infection indicates an immune system gone awry and manifests as excessive increased plasma concentrations of proinflammatory cytokines such as IL-1 $\beta$ , IL-2, IL-6, IL-10, granulocyte colony-stimulating factor, interferon- $\gamma$ -inducible protein 10, macrophage inflammatory protein 1 alpha, monocyte chemoattractant protein 1, and TNF- $\alpha$ .<sup>27</sup> The pro-inflammatory cytokines spread throughout the body, including the heart, through the systemic circulation, even as the cytokine storm occurs in local organs or tissues.<sup>5</sup> Thus, dysregulated immune and inflammatory function is important in COVID-19-related myocarditis.<sup>51</sup>

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

In the past few decades, understanding the critical role of the neuroimmune system has been enhanced by the clarification of the overlapping distributions and interaction between the nervous system and immune system in the regulation of immunological and inflammatory responses.<sup>35,36,69</sup> Among the neuroimmune interactions, the vagus nerve generated considerable interest when it was characterized as a major regulator of inflammation.<sup>31,32,70</sup> Borovikova and colleagues have demonstrated that acetylcholine (ACh), the principle vagal neurotransmitter, significantly attenuates inflammation in the human macrophage response to lipopolysaccharide (LPS).<sup>29</sup> An interesting and important finding is that the level of circulating TNF- $\alpha$  is significantly increased by bilateral cervical vagotomy in rats that were administered LPS but is markedly decreased when the distal end of the efferent vagus nerve is stimulated by constant voltage pulses in vagotomized rats that were administered LPS.<sup>29</sup> Therefore, the vagus nerve pathway, termed the CAP, plays a major role in the neural control of inflammation and the neuroimmune dialogue.<sup>29,31</sup>

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 through ACE-2. The principle vagal neurotransmitter ACh should bind ACh receptors, particularly  $\alpha 7$  nicotinic ACh receptors

( $\alpha 7$ nAChR), and consequently control immune cells and inhibit the production of inflammatory cytokines.<sup>66,71</sup> The  $\alpha 7$ nAChR is composed of five identical  $\alpha 7$  subunits and is the central component of the CAP that influences anti-inflammatory cells. It is encoded by *AHRNA7* on chromosome 15q14 and is widely expressed on the surfaces of inflammatory cells, including macrophages, monocytes, T cells, B cells, and dendritic cells.<sup>72–75</sup>

Wang and colleagues have found that the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 are significantly higher in endotoxemic  $\alpha 7$ nAChR<sup>-/-</sup> mice than wild-type mice.<sup>72</sup> When electrical stimulation is applied to the vagus nerve, endotoxin-induced serum TNF levels are significantly attenuated in wild-type mice but not in  $\alpha 7$ nAChR<sup>-/-</sup> mice.<sup>72</sup> Experiments in human macrophages and mouse peritoneal macrophages have further demonstrated that the release of TNF- $\alpha$  induced by LPS from macrophages is inhibited by Ach and nicotine through the stimulation of  $\alpha 7$ nAChR.<sup>72</sup> In monocytes,  $\alpha 7$ nAChR activated by non- or strong agonists downregulates NF- $\kappa$ B nuclear translocation and TLR4 expression and suppresses the transcription of pro-inflammatory cytokines.<sup>29,36,75</sup> De-Pu and colleagues<sup>76</sup> have used nicotine to activate  $\alpha 7$ nAChR 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  $\alpha 7$  subunit is essential for the cholinergic suppression of TNF- $\alpha$  and the normal regulation of systemic inflammatory responses.<sup>36,72</sup>

Recently, several studies have demonstrated that  $\alpha 7$ nAChR is an important mediator of nicotine's upregulation of ACE-2,<sup>77,78</sup> the main receptor used by SARS-CoV-2 to enter host cells.<sup>12,18,51</sup> The repurposing of  $\alpha 7$ nAChR antagonists has been proposed as a method to alter ACE-2 expression and prevent SARS-CoV-2 entry,<sup>77,78</sup> although the interaction between SARS-CoV-2 and CAP requires further investigation.<sup>79</sup> 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 the parasympathetic tone and cardiovascular regulation.<sup>66</sup> Therefore, targeting the CAP, particularly  $\alpha 7$ nAChR, through vagus nerve stimulation (VNS) might be a useful therapeutic measure for patients with COVID-19-related myocarditis.<sup>80</sup>

## 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,<sup>29,30,37</sup> dementia,<sup>38–41</sup> arthritis,<sup>35,36</sup> and cerebro- and cardiovascular diseases.<sup>81,82</sup> Koopman has found that the serum levels of TNF- $\alpha$ , IL-1 $\beta$ , 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.<sup>83</sup> In patients undergoing off-pump surgical revascularization, the peripheral blood production of TNF- $\alpha$  and IL-6 significantly decreases after 6 h of VNS treatment.<sup>84</sup> Additionally, some clinical trials that aim to assess the effects of VNS in diseases such as heart failure, atrial fibrillation, and traumatic brain injury are currently in progress.<sup>66</sup>

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.<sup>42,80,85</sup> Staats and coworkers<sup>34</sup> have reported that patients with COVID-19 show expedited symptomatic recovery from severe cough, chest tightness, and shortness of breath after noninvasive VNS. Leung<sup>78</sup> and Russo<sup>77</sup> have suggested that methyllycaconitine or  $\alpha$ -conotoxin, repurposed  $\alpha 7$ nAChR antagonists, are potential medications for

COVID-19, although this usage should be approached with caution.<sup>79</sup>

Importantly, the CAP has been shown to decrease the inflammatory response in viral myocarditis.<sup>76,86</sup> Activated  $\alpha 7$ nAChR by nicotine improves the balance of Th1/Th2 and Th17/Treg cell functional axis<sup>76</sup> and significantly downregulates the expression of TNF- $\alpha$  and IL-6 in mice with CVB3-induced viral myocarditis.<sup>86</sup> In addition,  $\alpha 7$ nAChR plays an important role in the expression of ACE-2, the target receptor of SARS-CoV-2.<sup>66,77,78</sup> 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 multiethnicity prospective clinical trials are needed to further extensively assess the anti-inflammatory effects of the CAP in COVID-19-related myocarditis.

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## 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.

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