

HYPOTHESIS ARTICLE - THEMED ISSUE

Dual inhibition of CB₁ receptors and iNOS, as a potential novel approach to the pharmacological management of acute and long COVID-19

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COVID-19 (SARS-CoV-2) causes multiple inflammatory complications, resulting not only in severe lung inflammation but also harm to other organs. Although the current focus is on the management of acute COVID-19, there is growing concern about long-term effects of COVID-19 (Long Covid), such as fibroproliferative changes in the lung, heart and kidney. Therefore, the identification of therapeutic targets not only for the management of acute COVID-19 but also for preventing Long Covid are needed, and would mitigate against long-lasting health burden and economic costs, in addition to saving lives. COVID-19 induces pathological changes via multiple pathways, which could be targeted simultaneously for optimal effect. We discuss the potential pathologic function of increased activity of the endocannabinoid/CB₁ receptor system and inducible NO synthase (iNOS). We advocate a polypharmacology approach, wherein a single chemical entity simultaneously interacts with CB₁ receptors and iNOS causing inhibition, as a potential therapeutic strategy for COVID-19-related health complications.

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COVID-19, iNOS inhibitor, peripheral CB₁ antagonist, polypharmacology, pulmonary fibrosis, SARS-CoV-2

1 | INTRODUCTION

Coronavirus disease (COVID-19) caused by the SARS-CoV-2 virus has emerged as a viral pandemic causing respiratory distress and pulmonary inflammation. Most cases of SARS-CoV-2 infection are

either asymptomatic or are associated with mild to moderate symptoms followed by a full recovery. However, a substantial percentage of infected individuals develop serious complications requiring intensive care unit hospitalisation and mechanical ventilation via tracheal intubation. In such patients, dyspnoea and hypoxaemia develop shortly after disease initiation that can lead to respiratory failure, acute respiratory distress syndrome (ARDS) and multi-organ failure.

Abbreviations: ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; CT, computed tomography; SARS-CoV-2, severe acute respiratory syndrome-coronavirus-2.

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2 | COVID-19: FROM RESPIRATORY INFECTION TO MULTIPLE METABOLIC HEALTH COMPLICATIONS

Earlier epidemiological surveys have shown that elderly patients or those with underlying chronic conditions such as obesity, chronic kidney disease, heart failure and/or hypertension were more susceptible to severe COVID-19 infection (Petrilli et al., 2020). In such patients, COVID-19-related ARDS may be accompanied by shock, heart failure, acute kidney injury and coagulation dysfunction (Petrilli et al., 2020). As additional features of the viral infection emerge, it appears that respiratory illness alone is no longer the critical factor in COVID-19 progression, as there are indications that virus-induced damage to the vascular endothelium underlies the formation of micro-clots in the lungs and the hypoxia observed in severely ill patients. The blood clots in other organ systems—mainly the brain, kidney and liver and even frostbite-like swollen wounds on fingers and toes—reflect endothelial impairment as the underlying process (Ackermann et al., 2020). This parallels the ‘cytokine storm’ and

leads to a ‘coagulation storm’, ultimately resulting in multi-organ failure and death in some patient populations (Ackermann et al., 2020). Obesity is known to increase pro-thrombotic factors and **leptin** production and hyperleptinaemia in obesity increase thrombotic risk (Konstantinides et al., 2001). Obesity and diabetes are known to be accompanied by metabolic inflammation. Although it has not been definitively established that inflammation is responsible for the increased COVID-19 morbidity and mortality in obese individuals, a case could be made for normalising obesity-related hyperleptinaemia as part of therapeutic management strategies in such patients. Additionally, elevated fasting blood glucose upon hospital admission has been highlighted as a predictor of poor prognosis and mortality among COVID-19 patients even in the absence of diabetes (Carrasco-Sanchez et al., 2021). Another study provided mechanistic insight on how increased glucose levels alter immune function to favour viral replication, induce monocytic cytokine production and T-cell dysfunction, which result in lung epithelial damage (Codo et al., 2020). These studies also highlight the importance of maintaining glucose homeostasis during the

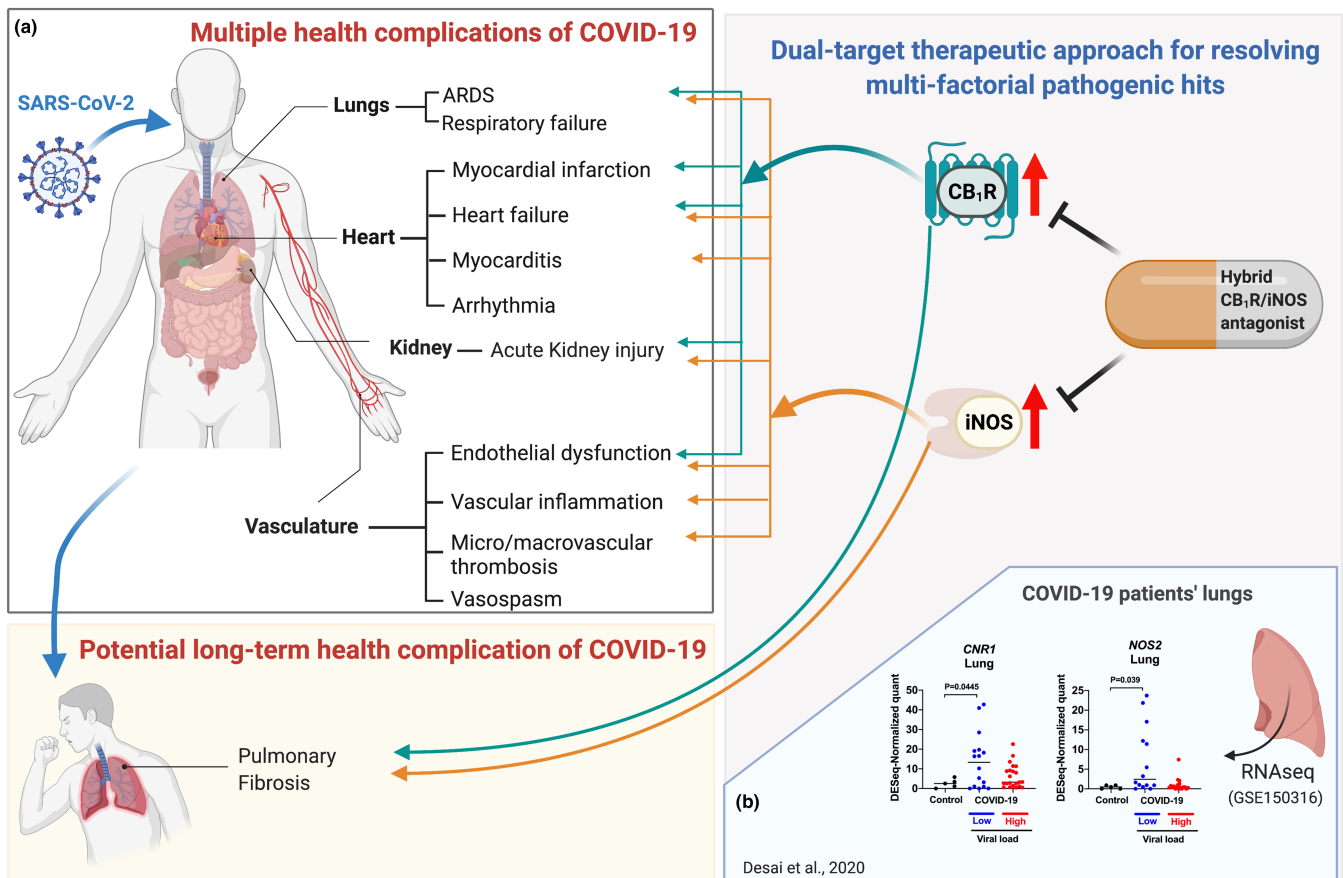


FIGURE 1 Multi-target therapeutic strategy for COVID-19-related multiple health complications by dual inhibition of CB₁ receptors and iNOS. (a) Dual inhibition of CB₁ receptors (CB₁R) and iNOS may not only attenuate acute COVID-19 complications such as acute respiratory distress syndrome (ARDS), septicaemia, heart failure, acute kidney injury, endothelial dysfunction and thrombosis but may also mitigate the development of pulmonary fibrosis as a late sequela of COVID-19. (b) Both CNR1 and NOS2 gene expression are increased in lungs of COVID-19 patients. Lung expression profiles of CNR1 and NOS2 were retrieved from RNAseq data set (Desai et al., 2020) from five virus-negative controls, seven COVID cases with low viral load and eight COVID cases with high viral load. Multiple data points obtained from different lung lobes were used for COVID cases considering temporal and spatial heterogeneity of host response to SARS-CoV-2 infection. The figure was generated in Biorender.com

progression of COVID-19 infection. Furthermore, blood vessel damage could also explain why pre-existing conditions like hypertension, obesity, diabetes and heart disease are risk factors for severe complications from SARS-CoV-2. All of those conditions cause endothelial cell dysfunction which could facilitate hyper-inflammation in the lungs as a result of the viral attack. Thus, COVID-19 is not only a respiratory disease but also a metabolic and inflammatory disease, and therefore therapeutic interventions should not be limited to mitigate acute damage caused by SARS-CoV-2, but should also aim to resolve many of the pathologies that develop as the viral infection progresses (Figure 1).

3 | FINDING CURE FOR ACUTE MANIFESTATIONS OF COVID-19: FROM SINGLE- TO MULTI-TARGET APPROACHES

Currently, a well justified race is underway globally to test a spectrum of candidate drugs in clinical trials for COVID-19. Therapeutic approaches involve repurposing earlier approved medications and generic drugs, as well as the use of promising, as yet unapproved, experimental small molecules. Corticosteroids such as **dexamethasone** and hydrocortisone have shown promise in critically ill patients. Antibody treatments that can contain the ‘cytokine storm’ in COVID-19, such as convalescent plasma therapy, are also being tested to combat the viral infection and have since gained emergency use authorisation. Operation Warp Speed supports the development and deployment of effective COVID-19 vaccines in record time. The history of infectious diseases such as HIV-AIDS suggests that it will take a ‘cocktail’ of drugs, that is, a combination therapy, to minimise the repercussions of the viral infection. Indeed, an approach to simultaneously impact multiple pathologic pathways has been adopted for COVID-19 (Hung et al., 2020). COVID-19 patients with underlying chronic diseases are already subjects to polypharmacy. In the present proposal, we advocate the use of a polypharmacology approach wherein a single chemical entity simultaneously engages two distinct targets to improve multiple conditions resulting from COVID-19 infection. A rational polypharmacology approach can also improve therapeutic efficacy and safety margin and would minimise limitations arising from the use of multiple chemical entities (Proschak et al., 2019), such as differential pharmacokinetics and target organ engagement, possible drug–drug interactions and potential adverse effects in a setting of compromised immune and organ function secondary to the viral attack.

4 | POTENTIAL FUTURE HEALTH COMPLICATIONS IN COVID-19 SURVIVORS: PULMONARY FIBROSIS?

Besides the acute health threat represented by COVID-19 including ARDS, septicaemia, coagulation dysfunction, kidney dysfunction and pulmonary embolism, there is also growing concern about potential

late complications among survivors of an acute COVID-19 infection. Recently emerging data document, the persistence of symptoms such as fatigue, dyspnoea and chest pain among the survivors of SARS-CoV-2 infection 2–3 months following recovery (Carfi et al., 2020). In the case of prior SARS-CoV epidemics, a substantial proportion of patients developed pulmonary fibrosis within a year of the acute infection (Hui et al., 2005). Based on lung computed tomography (CT) scans of recent COVID-19 patients and their 1-year follow up, there is evidence for pulmonary fibrosis development (Picchi et al., 2020; Tale et al., 2020; Ye et al., 2020). Therefore, pulmonary fibrosis could be a late consequence of COVID-19 (George et al., 2020; Spagnolo et al., 2020; Vasarmidi et al., 2020). Pulmonary fibrosis is a progressive disease, causing significant decline in pulmonary function and mortality within 3–5 years of the initial diagnosis. Currently, idiopathic pulmonary fibrosis is considered a rare disease with an estimated prevalence of 13 to 20 per 100,000 people worldwide. However, in a possible scenario of 20–25% of COVID-19 survivors developing pulmonary fibrosis, COVID-19-related pulmonary fibrosis would become a common disease affecting up to 120 million SARS-CoV-2 cases worldwide based on current infection levels. Therefore, it is important to understand the proliferative mechanisms that act as chronic pathological stressors in the lung of COVID-19 sufferers.

5 | INCREASED CB₁ RECEPTOR ACTIVITY INVOLVED IN OBESITY, DIABETES, STEATOSIS, DYSLIPIDAEMIA, HYPERLEPTINAEMIA AND SEPTIC SHOCK

Hyper-inflammation is attributed to the release of pro-inflammatory cytokines (e.g. **IL-6**, **IL-1**, **IL-8**, **TNF- α**), chemokines (**MCP-1**, **IP-10**) and scaffold molecules (NLRP3 inflammasomes) that may contribute to the rapidly developing systemic organ failure observed in some critically ill COVID-19 patients. In addition, co-morbidities like obesity, diabetes and hypertension can exacerbate the negative consequences of the viral attack. Many of the above pathologies involve an overactive endocannabinoid/**cannabinoid receptor 1 (CB₁)** system. Endocannabinoids are bioactive lipid signalling molecules that act via cannabinoid receptors, **CB₁** and **CB₂**. These are G-protein coupled receptor (GPCR) which mediate the effects of the psychoactive ingredients of cannabis. Although **CB₁** receptors are mainly found in the brain they are also widely distributed in peripheral tissues. Over-activation of the endocannabinoid/**CB₁** receptor system in peripheral organs contributes to obesity (Ruiz de Azua et al., 2017) and related metabolic disorders including type-2 diabetes (Jourdan et al., 2013), hepatic steatosis, dyslipidaemia, hyperleptinaemia and leptin resistance (Tam et al., 2010, 2012) and diabetic nephropathy (Jourdan et al., 2014). Conversely, peripheral **CB₁** receptor antagonism is effective in attenuating obesity and related metabolic complications (Cinar et al., 2020). **CB₂** receptors are expressed mostly in the periphery, primarily in immune cells, and their activation has anti-inflammatory properties in multiple inflammatory disorders (Maccarrone et al., 2015). Accordingly, **CB₂** receptor agonism may have therapeutic

benefit in COVID-19 by limiting inflammatory cytokine release, an effect discussed in recent review articles (Onaivi & Sharma, 2020; Rossi et al., 2020).

6 | CB₁ RECEPTORS INVOLVED IN INFLAMMATORY DISORDERS: LUNG INFLAMMATION, ARDS, INCREASE VIRAL LOAD, SEPSIS, ACUTE KIDNEY INJURY AND ORGAN FIBROSIS

Recent observations show that endocannabinoids acting via CB₁ receptors are induced in certain viral infections and exacerbate viral loads (Reiss, 2010; van der Poorten et al., 2010). In turn, CB₁ receptor blockade has been shown to exert anti-viral effects by repressing viral replication and to protect from or reverse sepsis (Shahidi et al., 2014; Singh et al., 2018). Activation of peripheral CB₁ receptors also contributes to septic hypotension whereas CB₁ receptor blockade protects against endotoxin-induced hypotension (Batkai et al., 2001; Varga et al., 1998) and lung injury (Johnson et al., 2015). Additionally, CB₁ receptor up-regulation by potent synthetic cannabinoids is directly linked to hypoxia and acidosis leading to ARDS and respiratory failure (Alon & Saint-Fleur, 2017). CB₁ receptor-induced lung injury and inflammation are mediated through **IL-1 β** , TNF- α , IL-6, MCP-1, IRF5 and NLRP3 signalling (Zawatsky et al., 2020), which lead to pro-inflammatory and pro-fibrotic sequelae (Cinar et al., 2017). Furthermore, activating renal and cardiac CB₁ receptors induces nephropathy (Jourdan et al., 2018; Udi et al., 2017) and cardiomyopathy (Batkai et al., 2007; Rajesh et al., 2012), respectively. Additionally, use of synthetic cannabinoids results in a high rate of acute kidney injury and cardiac dysfunction (Pacher et al., 2018; Tam, 2016). CB₁ receptor activation contributes to organ fibrosis in liver, lung, heart and kidney (Cinar et al., 2020). All this makes CB₁ receptors plausible therapeutic targets in metabolic, inflammatory and fibroproliferative disorders.

Indeed, the first-in-class, globally acting CB₁ antagonist/inverse agonist **rimonabant** had beneficial effects in metabolic syndrome (Despres et al., 2005) and was consequently approved as an anti-obesity drug in about 40 countries. However, psychiatric side effects due to blockade of CB₁ receptors in the central nervous system resulted in its withdrawal from the clinic and halted the therapeutic development of related brain-penetrant CB₁ antagonists. Increasing evidence in the last decade has shown that non-brain-penetrant CB₁ antagonists retain the metabolic benefit of globally acting compounds without their adverse behavioural effects and show therapeutic potential in preclinical models of obesity and related metabolic disorders (Cinar et al., 2020).

7 | INDUCIBLE NITRIC OXIDE SYNTHASE: INFLAMMATION, ARDS, SEPSIS AND ORGAN FIBROSIS

Another potential therapeutic target that parallels the role of CB₁ receptors in many pathologies is **inducible nitric oxide synthase**

(**iNOS**), an enzyme that catalyses the generation of pro-inflammatory reactive nitrogen species. Increased expression of iNOS in the lung was detected along with IL-6 and IL-8 in ARDS patients following sepsis, suggesting that iNOS along with other pro-inflammatory mediators produced by alveolar macrophages play an important role in the pathogenesis of acute lung injury (Kobayashi et al., 1998). iNOS expression in the TNF- α /iNOS pathway was also prominent in malaria infection-induced ARDS (Galvao-Filho et al., 2019). iNOS plays a key role in septic shock as mice lacking iNOS have been reported to be resistant to endotoxin-induced mortality (Vincent et al., 2000). Similarly, iNOS inhibitors have protective effects in various forms of septic shock and inflammation (Southan et al., 1995). The effect of selective iNOS inhibition has been tested in clinical trials for sepsis/endotoxaemia. In humans, proximal tubular injury during systemic inflammation is correlated with up-regulation of renal iNOS. Hence, the selective inhibition of renal iNOS may resolve sepsis-induced acute kidney injury (Heemskerck et al., 2009).

Acute lung inflammation and sepsis also lead to cardiovascular complications such as atherosclerotic plaque destabilisation characterised by intraplaque haemorrhage and thrombus (Jaw et al., 2016), which was also the case in COVID-19 patients (Guzik et al., 2020). iNOS inhibition prevented atherosclerotic plaque destabilisation in **lipopolysaccharide (LPS)**-induced acute lung injury (Moritani et al., 2017). Furthermore, inhibition of iNOS attenuates immune complex-induced vascular injury in rat lungs and skin (Tripathi et al., 2007). iNOS inhibitors also repair myocardial contractility in hearts exposed to pro-inflammatory cytokines (Xue et al., 2018).

The above suggest that iNOS inhibition could offer therapeutic benefit in COVID-19-related acute lung injury, cardiovascular complications such as thrombosis and atherosclerotic plaque destabilisation and sepsis-induced organ injury such as acute kidney injury.

8 | STATUS OF CB₁ RECEPTOR AND INOS EXPRESSION IN LUNGS OF COVID-19 PATIENTS

A recent study reported gene expression profiles in the lungs of patients who had died of COVID-19 and in five COVID-19 negative controls, using RNAseq (Desai et al., 2020). Analysis of the data retrieved from the Omnibus (GEO) database, accession number GSE150316, indicated significant increases in **CNR1** and **NOS2**, encoding CB₁ receptor and iNOS, respectively, in COVID-19 lungs with low viral load compared to non-COVID-19 controls (see Figure 1). Additionally, iNOS protein expression was also significantly increased in lungs of COVID-19 patients relative to controls (Chen et al., 2021). The current evidence warrants further investigations of the potential pathologic role of CB₁ receptor and iNOS in COVID-19 induced lung disease and the therapeutic potential of inhibitors of CB₁ receptors and iNOS.

9 | DUAL INHIBITION OF PERIPHERAL CB₁ RECEPTOR AND INOS FOR ACUTE AND LONG-TERM HEALTH COMPLICATIONS OF COVID-19

In view of the findings described above, overactivation of iNOS and increased CB₁ receptor signalling may be involved in the acute manifestations of COVID-19. Although peripheral CB₁ receptor antagonism or iNOS inhibition alone may have promise as therapeutic modalities for multiple pathologies associated with COVID-19, a combined approach may be superior. Combined inhibition of iNOS and CB₁ receptors may tame the potentially lethal cytokine storm and severe lung inflammation in COVID-19 patients. Simultaneous abrogation of CB₁ receptor and iNOS pathways may also attenuate clot formation and thrombosis, increasingly seen in severe COVID-19 infection, septic shock, sepsis-induced multi-organ dysfunction such as cardiomyopathy and acute kidney injury, as discussed above.

Beside acute complications, there is a major concern regarding the development of pulmonary fibrosis in some COVID-19 survivors (Spagnolo et al., 2020), although the true prevalence of pulmonary fibrosis following COVID-19 will not be established for months or years. Both CB₁ receptor and iNOS overactivation promote alveolar inflammation, pulmonary fibrosis initiation and progression (Cinar et al., 2017; Romanska et al., 2002). Simultaneous inhibition of CB₁ receptors and iNOS by a hybrid inhibitor resulted in greater anti-fibrotic effect than did inhibition of either target alone in experimental studies using hybrid CB₁ receptor/iNOS inhibitor, **MRI-1867** (Cinar et al., 2017). Whereas dual CB₁ receptor/iNOS inhibition may attenuate acute respiratory and metabolic complications of COVID-19, it remains to be seen whether such treatment also mitigates pulmonary fibrosis development among COVID-19 survivors? In summary, the combined pharmacological inhibition of CB₁ receptors and iNOS has promise for limiting severe organ damage and improving patient outcomes in COVID-19.

10 | CONCLUSION

COVID-19 leads to serious complications that require early recognition and comprehensive management. Based on the documented involvement of increased CB₁ receptor and iNOS activity in multiple complications of severe COVID-19 infection, their simultaneous inhibition by a single chemical entity may have considerable therapeutic potential, which needs to be validated in the clinic once a candidate drug becomes available for human use. The strategy outlined can serve as a viable complement to anti-viral drugs against COVID-19.

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AUTHOR CONTRIBUTIONS

R.C., M.R.I. and G.K. wrote the manuscript.

CONFLICT OF INTEREST

R.C., M.R.I. and G.K. are listed as co-inventors on US patents covering hybrid CB₁ receptor/iNOS antagonists.

DATA AVAILABILITY STATEMENT

No data have been shared.

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