Rationale for Evaluating PDE4 Inhibition for Mitigating against Severe Inflammation in COVID-19 Pneumonia and Beyond

Charlie Bridgewood PhD[1], Giovanni Damiani MD[3,4], Kassem Sharif MD[1,5], Abdulla Watad MD[5,6], Nicola Luigi Bragazzi MD PhD MPH[7,8], Luca Quartuccio MD[9], Sinisa Savic MD[1,10] and Dennis McGonagle FRCPI PhD[1,2]

[1]Department of Musculoskeletal Disease, Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK
[2]National Institute for Health Research (NIHR), Leeds Biomedical Research Centre (BRC), Leeds Teaching Hospitals, Leeds, UK
[3]Department of Clinical Dermatology, IRCCS Galeazzi Orthopedic Institute, Milan, Italy
[4]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy
[5]Department of Medicine B and [6]Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel Hashomer, Israel
[7]Department of Health Sciences (DISSAL), School of Public Health, University of Genoa, Genoa, Italy
[8]Laboratory for Industrial and Applied Mathematics (LIAM), Department of Mathematics and Statistics, York University, Toronto, Canada
[9]Clinic of Rheumatology, ASUFC, Department of Medicine (DAME), University of Udine, Udine, Italy
[10]Department of Clinical Immunology and Allergy, St James's University Hospital, Leeds, UK

ABSTRACT In the absence of definitive anti-viral therapy, there is considerable interest in mitigating against severe inflammatory reactions in coronavirus disease-2019 (COVID-19) pneumonia to improve survival. These reactions are sometimes termed cytokine storm. PDE4 inhibitors (PDE4i) have anti-inflammatory properties with approved indications in inflammatory skin and joint diseases as well as chronic obstructive pulmonary disease. Furthermore, multiple animal models demonstrate strong anti-inflammatory effects of PDE4i in respiratory models of viral and bacterial infection and also after chemically mediated lung injury. The rationale for PDE4i use in COVID-19 patients comes from the multimodal mechanism of action with cytokine, chemokine, and other key pathway inhibition all achieved with an excellent safety profile. The authors highlight how PDE4i could be an overlooked treatment from the rheumatologic and respiratory armamentarium, which has potential beneficial immune-modulation for treating severe COVID-19 pneumonia associated with cytokine storms. The proposed use of PDE4i is also supported by age-related immune changes in inflammation severity in PDE4i modifiable pathways in primate coronavirus disease. Over-exuberant anti-viral immune responses in older patients with COVID-19 may pose a substantial risk to patient survival and mitigation against such hyper-inflammation with PDE4i, especially with anti-viral agents, is a strategy that need to be pursed, especially in older patients.

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Severe coronavirus disease-2019 (COVID-19) infections are characterized by pneumonia, lymphopenia, functional exhaustion of lymphocytes, and a prominent cytokine storm that is also termed as a macrophage activation syndrome-like state [1]. Prior to the COVID-19 pandemic, it was known that other corona viruses, including severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), Middle Eastern respiratory syndrome CoV (MERS-CoV), and even fatal influenza H7N9 virus infection, were often associated with severe pulmonary inflammation with systemic hypercytokinemia [2-5]. There is also some evidence that the severe inflammatory reactions that may accompany viral infection may be secondary to the viral load and thus immune responses may ultimately be beneficial and not harmful [6,7]. Accordingly, cytokine blockade in COVID-19 cases with active infection and both type-1 interferon pathway suppression, in addition to lymphopenia, is completely different for cytokine antagonism in settings such as CAR-T cell therapy or adult-onset Still's disease related macrophage activation syndrome (MAS) [8].

Unlike COVID-19 pneumonia, the latter two scenarios may occur in the setting of complete immunocompetence or even immune system gain of function, so cytokine blocking strategies are reasonable. However, the same strategies might be harmful in some COVID-19 cases due to relative immunodeficiency [8]. The situation is further complicated by our inability to currently evaluate active infection and viral loads that might be useful in directing anti-cytokine strategies [9].

Since the outset of the COVID-19 pandemic, at least 10 drugs, including anti-cytokine therapy and DMARDs approved for the therapy of the rheumatic diseases, have been suggested as potential targets to reduce COVID-19-related mortality [10,11]. A major problem with severe COVID-19 pneumonia is virally induced lymphopenia and the blunted type 1 interferon responses

[12,13]. With the exception of hydroxychloroquine, all of the proposed anti-rheumatic agents are associated with an increased risk of infections, especially respiratory infections, in patients who otherwise have normal pulmonary homeostasis. Given hydroxychloroquine's proven efficacy in diseases such as lupus, where interferon signatures are high prior to therapy, additional blunting of interferon responses in COVID-19 disease may be undesirable.

Many immunomodulatory or immunosuppressive therapies have been proposed to treat the hyperinflammation associated with COVID-19 pneumonia [14]. We have highlighted how the severe inflammation in COVID-19 pneumonia is not typical of MAS that is seen in rheumatology, hematology, or immunology but represents a pulmonary MAS-like state [8]. We have also drawn attention to how such inflammation driven immunothrombosis may drive a pulmonary microvascular coagulopathy and that this represents an overlooked lung-based hyper-inflammatory infection related state that is distinct from any rheumatological entity such as MAS [15].

There has been an emphasis on the use of targeting single cytokines or multiple cytokine pathways with drugs that can increase the risk of infection in healthy people, not to mention the implications of such therapy in COVID-19-induced type-1 interferon pathway abrogation, lymphopenia, or lung damage and the recognized development of secondary superinfections. With failed type-1 interferon responses it is likely that a second wave of pro-inflammatory cytokines rather than one specific molecule drives the severe inflammatory cascade in older patients. Therefore, an argument for strategies that target several inflammatory pathways without completely abrogating anti-viral-interferon strategies with therapies with good safety profile is needed. The phosphodiesterase 4 inhibitors (PDE4i) apremilast is a rheumatology therapy that may meet these requirements. In parallel therapy developments, PDE4i are also approved in respiratory medicine for chronic obstructive pulmonary disease (COPD) with roflumilast being available with this agent representing a second available agent that could be repurposed [16].

Since PDE4i mitigate against pulmonary, joint, and skin inflammation, we suggest harnessing this proven role in the lung arena to mitigate against the severity of COVID-19-related pneumonitis with associated immunothrombosis in older patients. Presumably, the slow onset of action compared to anti-cytokine blockers in the face of a severe viral pneumonia may be a principle reason for lack of interest in these agents. Nevertheless, the idea of early use and prophylaxis against viral pneumonia evolution in at risk groups is worthy of consideration, especially given the safety and a comparative strong therapeutic rationale compared to some other agents. Also, these agents could be used at higher doses, the most common dose limiting factor being gastrointestinal intolerance. This treatment could be of particular value in relatively forgotten COVID-19 hotspots including residential care facilities. Given the emerging data showing that antiviral agents, especially remdesivir, reduces time to recovery but not mortality in COVID-19 pneumonia, it would seem advisable to use PDE4i as an add-on therapy rather than in isolation.

PDE4 inhibitors are small molecule inhibitors used to treat a range of inflammatory diseases, including psoriasis, psoriatic arthritis, and chronic obstructive pulmonary disease

Rationale for PDE4 inhibitors

The PDE4 enzymes are the main cyclic adenosine monophosphate (cAMP) degrading enzymes. The principle mechanism of PDE4i is to increase intracellular levels of cAMP. cAMP is a key intracellular second messenger, and its effects are transduced by two ubiquitously expressed intracellular cAMP receptors: protein kinase A and exchange protein directly activated by cAMP [17,18]. cAMP is able to inhibit activation of the NF-kβ pathway, and thus the production of inflammatory mediators [17]. PDE4 is present in a range of cells including, lymphocytes, monocyte/macrophages, granulocytes, fibroblasts, and epithelial cells. Accordingly, PDE4i have the ability to attenuate, but not completely block, various key inflammatory mediators including tumor necrosis factor (TNF), interleukin-12 (IL-12), IL-17, and several chemokines [19]. The most common PDE4i are apremilast (psoriasis and psoriatic arthritis) and those used for the management of COPD (cilomilast and roflumilast). The rationale for the potential use of PDE4i for treating COVID-19 can be divided between in vivo/in vitro evidence and knowledge gained from PDE4i use in psoriasis and COPD.

Lessons from the clinic including pulmonary pathology with PDE4i

When considering the potential benefit of PDE4i for COVID-19 infection, it is important to evaluate what is known about fPDE4i in the context of lung inflammation and indeed PDE4i lead to improvements in the spirometry and exacerbations of COPD patients. The exact mechanisms behind PDE4i in improving symptoms is unknown in COPD, but PDE4i does reduce the amount of pulmonary infiltrating neutrophils and lymphocytes [20,21]. Roflumilast use is not associated with an increase in upper respiratory tract infection or (URTI) influenza [16]. Similarly, apremilast is not associated with an increase in viral UTRI when used for psoriasis or psoriatic arthritis [22]. Like many other viral infections, COVID-19 neutralizes type-1 interferon responses and apart from partial attenuation of interferon-β signaling in the in vitro setting there is no obvious mechanistic downside of PDE4i [23].

Pathology of COVID-19-induced severe pulmonary inflammation and PDE4

The pathology of COVID-19 pneumonia includes prominent T cell, myeloid, and neutrophilic infiltration of alveoli and interstitium [24,25]. The most severe COVID-19 cases have elevation in many of the cytokines known to be inhibited by PDE4i, such as TNF and IL-1β [1]. Emergent single cell RNA sequences from bronchoalveolar lavage fluid show activated macrophages with expression of several chemokines, all of signal via G protein-coupled receptors (GPCRs) with cAMP as the second messenger [26]. Of considerable importance is that coronavirus inflammation is more severe in older people and also in older monkeys in experimental settings [27]. Among the most up-regulated molecules in this model is IL-8 and tissue factor, which likely play a major role in COVID-19- related immunothrombosis.

Inhibitors have shown promise, in animal models, of bacterial/ viral respiratory infection and of pulmonary injury models

The in vivo and in vitro case to support PDE4 inhibition in COVID-19

Since the advent of the original PDE4i rolipram in the1990s numerous in vivo and in vitro studies have been conducted on PDE4i in acute and chronic lung inflammation. Regarding the lung, numerous animal models support the beneficial effect of PDE4i in respiratory inflammation. Although no animal model regarding COVID-19 or the related SARS exists for PDE4i, positive effects in other respiratory viral models such as influenza have been demonstrated following PDE4i, which is summarized in Table 1.

A strength of PDE4i for COVID-19 may be with its ability to control neutrophil-mediated responses. In rat model of coronavirus infection, neutrophils were required to mediate disease [21]. Neutrophils are major players in the immunopathology of COPD. Treatment of COPD patients with either cilomilast or roflumilast reduced the number of lymphocytes and neutrophils recruited to the lung in addition to important chemokines associated with neutrophil recruitment, such as IL-8 [20,21]. Studies have also shown that PDE4i is able to prevent neutrophil migration/chemotaxis into lungs and also adherence to the endothelium [28]. Apremilast has shown positive evidence for treating neutrophilic dermatoses such as SAPHO and hidradenitis suppurativa, in addition to psoriasis in which there is prominent neutrophilic inflammation. Macrophage infiltration has also been reported in lungs of COVID-19 patients. PDE4i has well-documented anti-inflammatory effects on alveolar macrophages, attenuating cytokine secretion [29].

Table 1. Summary of pulmonary animal models with PDE4i.

Animal model	Key findings	Reference*
Mice infected with Influenza H1N1 virus	PDE4i reduced lung inflammation PDE4i Reduced mortality	Sharma et al, 2013, Emerging microbes and infections
Guinea-pigs were inoculated with parainfluenza type 3	PDE4i reduced airway inflammation PDE4i lowered number of infiltrating monocytes and neutrophils	Toward et al, 2005, International immunopharmacology
Chlorine-induced lung injury of mice	PDE4i inhibited chlorine-induced pulmonary edema	Chang et al, 2012, Toxicology and applied pharmacology
Murine acute lung injury model-LPS and Zymosan treated mouse model	PDE4i protects against lung injury	Miolta et al, 1998, American journal of respiratory cell and molecular biology
Rats infected with LPS	PDE4i reduced alveolar hemorrhage	Turner et al, 1993, Circulatory shock
Murine LPS model	PDE4i reduced neutrophil migration to the lung	Ariga et al, 2004, Journal of Immunology
Ferret LPS model	PDE4i reduced lung neutrophilia	Schafer et al, 2014, Cellular signaling
Guinea pig model LPS induced lung neutrophilia	PDE4i inhibited 63% neutrophil infiltration	Toward and Broadley, 2001, Journal of Pharmacology and Experimental Therapeutics
Bleomycin induced lung inflammation in rats and mice	PDE4i ameliorated pulmonary inflammation and fibrosis	Pan et al, 2009, Respirology Cortijo et al, 2009, British Journal of pharmacology
Rat model of hyperoxia-induced lung injury	PDE4i reduced inflammation PDE4i prolonged survival	de Visser et al, 2008, European Respiratory Journal
Mouse model of Streptococcus pneumonia	PDE4i reduced lung injury PDE4i reduced neutrophil recruitment	Tavares et al, 2016, American Journal of Respiratory Cell and Molecular Biology

*References are not included in the Reference section
LPS = lipopolysaccharide, PDE4i =phosphodiesterase 4 inhibitor

PDE4i are also able to directly attenuate cytokine and chemokine secretion from a range of cells important in viral pulmonary pathology, such as macrophages, T-cells, fibroblasts, and epithelial cells. These also include TNF, IL-1β, IL-12, IL-23, IL-17, CCL2, IL-8, GM-CSF, and RANTES [19,29-31] [Figure 1]. Although PDE4i directly lower the production of numerous cytokines, PDE4i may also indirectly lower other cytokines, such as IL-6 and IL-8, by logic that there is less TNF or IL-17 to induce these. Both of these cytokines have been shown to be lower in lung injury models following PDE4i treatment [32]. With respect to structural integrity, elevating cAMP attenuates viral induced epithelial barrier damage [33]. Thrombotic complications are being widely reported the pathogenesis of COVID-19 infection [34], which is another area of potential benefit for PDE4i. Roflumilast inhibits leukocyte-platelet interactions and also the pro-thrombotic functions in leukocytes [35]. In a murine model, mimicking stroke, rolipram was demonstrated to be anti-thrombotic [36].

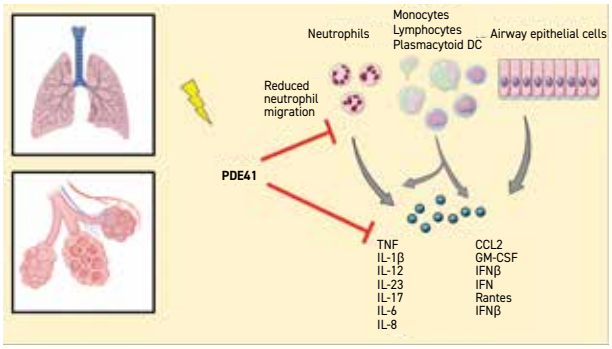
Regarding secretion of cytokines and chemokines, PDE4i have been shown to attenuate a range of important mediators also important in anti-viral response, which may be a potential downside. With respect to viral induced inflammation, the most important of these may be type 1 interferons. Plasmacytoid dendritic cells (pDC) are known to rapidly produce interferon-α in response to viral pathogens, including SARS [37] and have a role in pulmonary viral defense. Apremilast was shown to partially inhibit interferon-α from pDC following stimulation [19]. Interferon -β secretion has also been shown to be attenuated by elevating cAMP [23], while rolipram is able to reduce interferons-γ-inducible protein 10 (IP10) in a lung epithelial-PBMC co-culture model [38].

Of note, both severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 utilize the angiotensin receptor converting enzyme-2 (ACE2) for host cell entry and the serine protease TMPRSS2 for S protein priming [39]. The angiotensin-II molecule is metabolized into angiontensin-1-7 peptide, which exerts anti-inflammatory via engagement of the MAS receptor, which signals via GPCR and is thus potentially modifiable with PDE4i [40]. Given that PDE4i regulates cAMP, a key second messenger with many other potential targets, there are many other potential mechanisms that could be regulated and the net impact needs assessment.

Additional considerations

Severe inflammation in COVID-19 pneumonia might reflect the magnitude of the inflammatory reaction that is commensurate to the ongoing viral insult in the face of blunted type 1 interferon response and blunted adaptive immunity with viral associated lymphopenia. Therefore, just giving drugs or repurposing drugs on the basis of elevated inflammation in COVID-19 needs to be carefully balanced with therapy of ongoing viral infection. In that regard, anti-viral agents are available with some emerging efficacy data. Allowing for this treatment, the PDE4i agents have not been associated with exacerbations of viral infections.

Figure 1. The potential role of PDE4i in COVID-19 pulmonary infection. PDE4i reduces migration of neutrophils, monocytes, and lymphocytes to the inflamed lung. PDE4i reduces inflammatory cytokine and chemokine production from immune cells and airway epithelial cells



Conclusions

Several questions over PDE4i and COVID-19 at a mechanistic level still remain. Chief among these is the fast-evolving pathology of COVID-19 and where a small oral PDE4i with a narrow therapeutic index and no loading dose strategy can work quickly enough. Given the safety profile of PDE4 inhibitors it could represent a useful agent to test in carefully controlled trials for prophylaxis against most severe infection in older patients including nursing home residents who have been forgotten by clinical trials. This class of drugs may have a role in the elderly or those at risk for higher degrees of systemic inflammation that are linked to immunothrombosis. Indeed, older age and increased mortality following viral infection is well recognized and the current pandemic has likely crystallized a wider problem that is cases with severe and perhaps excessive inflammation could be usefully modified.

Due to their efficacy in treating pulmonary inflammation in chronic obstructive pulmonary disease, PDE4 inhibitors may be considered for COVID-19 infection

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Correspondence

Dr. C. Bridgewood
Dr. D. McGonagle
Dept. of Musculoskeletal Disease, Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, LS7 4SA, UK
Phone: (44-113) 392-4883
Fax: (44-113) 392-4991
email: medcbri@leeds.ac.uk; meddgm@leeds.ac.uk

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