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OPINION ARTICLE

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Targeting the NO-cGMP-PDE5 pathway in COVID-19 infection. The DEDALO project

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Abstract

Background: A pandemic outbreak of COVID-19 has been sweeping the world since December. It begins as a respiratory infection that, mainly in men with diabetes or renal impairment, evolves into a systemic disease, with SARDS, progressive endothelial cell damage, abnormal clotting and impaired cardiovascular and liver function. Some clinical trials are testing biological drugs to limit the immune system dysregulation, "cytokines storm," that causes the systemic complications of COVID-19. The contraindications of these drugs and their cost raise concerns over the implications of their widespread availability.

Objectives: Numerous clinical and experimental studies have revealed a role for the nitric oxide (NO)-cyclic GMP-phosphodiesterase type 5 (PDE5) pathway in modulating low-grade inflammation in patients with metabolic diseases, offering cardiovascular protection. PDE5 inhibition favors an anti-inflammatory response by modulating activated T cells, reducing cytokine release, lowering fibrosis, increasing oxygen diffusion, stimulating vascular repair. PDE5 is highly expressed in the lungs, where its inhibition improves pulmonary fibrosis, a complication of severe COVID-19 disease.

Materials and methods: We performed a systematic review of all evidence documenting any involvement of the NO-cGMP-PDE5 axis in the pathophysiology of COVID-19, presenting the ongoing clinical trials aimed at modulating this axis, including our own "silDEnafil administration in DiAbetic and dysmetaboLic patients with COVID-19 (DEDALO trial)."

Results: The reviewed evidence suggests that PDE5 inhibitors could offer a new strategy in managing COVID-19 by (i) counteracting the Ang-II-mediated down-regulation of AT-1 receptor; (ii) acting on monocyte switching, thus reducing pro-inflammatory cytokines, interstitial infiltration and the vessel damage responsible for alveolar hemorrhage-necrosis; (iii) inhibiting the transition of endothelial and smooth muscle cells to mesenchymal cells in the pulmonary artery, preventing clotting and thrombotic complications.

Discussion and Conclusion: If the ongoing trials presented herein should provide positive findings, the low cost, wide availability and temperature stability of PDE5

inhibitors could make them a major resource to combat COVID-19 in developing countries.

KEYWORDS

PDE5 inhibitors, cytokine storm, IL-6, interstitial pneumonia, type 2 diabetes mellitus, pulmonary fibrosis

1 | INTRODUCTION

COVID-19 is now a worldwide pandemic, with exceeded 2 millions of confirmed cases worldwide and a high mortality rate among the minority of people with COVID-19 who get severe disease (60.5% mortality for critical cases).[1-3]

Up to 20% of infected patients develop severe pneumonia evolving into severe acute respiratory syndrome (SARS), ending with multiple organ failure.[1,3,4] The two most frequent comorbidities in deceased patients were diabetes mellitus and hypertension.[5] This is extremely important given that over 463 million people worldwide have diabetes.[6]

Data from the Italian cohort highlight a significantly higher number of infections (approximately 4:1) and a higher fatality in men than in women: 8% of men died, compared with 5% of women (ISS - Higher Health Institute of Rome analysis of 25 058 cases)(Integrated surveillance of COVID-19 in Italy, https://www.epicentro.iss.it/coronavirus/).

The clinical worsening is apparently related to a hyperinflammation with excessive release of proinflammatory cytokines (TNF-alfa, IL-6), the cytokine storm, culminating in loss of control, immunosuppression, reduction in memory CD4+ T helper cells[7-10] and severe lung damage.[11] This may subsequently evolve to fibrosis, a potential sequela even after viral propagation has attenuated.

Comorbidities play a role in hampering the initial protective immune response, helping the virus spread, and facilitating the more severe phase through their previous dysregulation of the immune system.

The current designed protocols, including the use of chloroquine and tocilizumab for proinflammatory cytokine modulation, have significant side effects.[12,13]

In developing a new therapeutic strategy, it is reasonable to attempt to boost immune response during the first immune-mediated phase while suppressing the final cytokine-mediated phase,[8,14,15] preventing progression from mild to severe form and mitigating fibrosis in patients overcoming the acute stage.[16,17]

Androgen sensitivity could be a determinant of COVID-19 disease severity. The androgen sensitivity model might explain why males are more likely to develop severe symptoms whereas children seem to be resistant to infection. Androgen sensitivity is determined by genetic variants of the androgen receptor which regulates transcription of the transmembrane protease, serine 2 (TMPRSS2). This is required for SARS-CoV-2 infectivity: Cell entry of SARS-CoV-2 depends on binding of the viral spike proteins to ACE2. ACE2 is a functional receptor for SARS-CoV-2.[18] TMPRSS2 primes the Spike protein of the virus, which has two consequences: diminishing viral recognition by neutralizing antibodies and activating SARS-CoV-2 for virus-cell fusion.[19,20]

This has led to the intuition that TMPRSS2 inhibition may work to block or decrease the severity of SARS-CoV-2 infections and a recent study on almost 5 thousands COVID-19 patients demonstrated that those receiving androgen-deprivation therapy (ADT) had a significantly lower risk of SARS-CoV-2 infection compared with patients who did not receive ADT (OR 4.05).[20]

Given the presence of ACE2 receptor in testes also, COVID-19 infectious-related orchitis was examined and reported.[21] Although the presence of SARS-CoV-2 RNA in semen samples was reported in some,[22] but not all the studies,[23] data provide potential clues for further medical pathogenesis of COVID-19-related male infertility in infected patients.

The use of nitric oxide (NO) to treat COVID-19-related interstitial lung disease was approved by the FDA a few weeks ago (as reported by Bellerophon CEO Fabian Tenenbaum to BioWorld),[24,25] as already described in literature.[26,27]

Phosphodiesterase type-5 inhibitors (PDE5i) such as sildenafil were approved 20 years ago for the treatment of male erectile dysfunction, but they were originally tested as alternatives to nitrates for the relief of angina, as they enhance the nitric oxide/cyclic guanosine monophosphate (NO/cGMP) signaling pathway[28,29] with pleiotropic systemic effects.

Two different meta-analyses on chronic oral use of different formulation of PDE5i have shown reduced mortality[30] and a good safety profile[31] in patients with type 2 diabetes mellitus (T2DM) and high cardiovascular risk, endorsing the beneficial cardioprotective properties and inflammatory cytokine modulation previously demonstrated in the CECSID trial after chronic (3 months) oral sildenafil (100 mg/daily) on T2DM patients with diabetic cardiomiopathy.[32,33] To note, as hypogonadism frequently complicates T2DM resulting in severe endothelial dysfunction, in these context PDE5i efficacy on sexual function could be reduced, given the testosterone regulation of PDE5 expression as one of the major mechanisms controlling vasodilator mechanisms in penile tissue.[34-38]

Aside from pre-clinical studies demonstrating respiratory and renal protective effects of PDE5i,[39,40] both acute[41] and chronic administration of oral sildenafil at different dose in human have confirmed the benefit on pulmonary compliance as well as anti-remodeling effects,[31,39,40,42-44] especially in T2DM, where they

improve the endothelial dysfunction that underpins various metabolic, cardiovascular and inflammatory diseases.

Chronic (6 months) PDE5 inhibition through vardenafil 10 mg/die has been shown to preserve endothelial function in T2DM patients,[45] probably due to an increase in angiogenic mediators, including Ang1.[46] These effects seem particularly important given that neutrophils isolated from patients with T2DM release larger amounts of proinflammatory cytokines, above all TNF-α and IL-6,[47] main players of the cytokine storm seen in COVID-19 infection.

In mouse models of diabetes, sildenafil restores normal levels of circulating TIE2-expressing monocytes (TEMs), limiting inflammation, and promoting tissue repair.[46-48] These findings were then confirm in T2DM patients where 3 months of oral sildenafil (100 mg/day) reduced the endothelial function marker P-selectin.[49] In pre-clinical, as well as in clinical studies using chronic PDE5i with sildenafil 100 mg daily, we and others have shown that the NO-cGMP-PDE5 pathway and related targets are involved in the pathogenesis of several complications of diabetes affecting the immune system,[46-48] adipose tissue,[50,51] and renal function.[39] A dose of 50mg of Sildenafil, given twice a week, was shown to improve surrogate markers of endothelial function (C-reactive protein, endothelin-1 and ICAM-1, ANG-1/TIE2 axis), modulating the number of circulating proangiogenic cells and exerting an anti-inflammatory response in T2Dm patients.[52]

PDE5 is predominantly expressed in the lungs, the organ most affected by COVID-19.

Its expression in men[53] is even higher, making them more responsive to PDE5i, as revealed in pulmonary hypertension (PH) and fibrosis.[54-56] Sildenafil is approved for the treatment of PH and is currently indicated in patients with WHO functional class II and III PH. The approved posology is 20 mg three times per day. The use of PDE5i to improve endothelial function by the NO-cGMP pathway could help repair alveolar-vascular interface damage,[57] thus improving O2 diffusion, as demonstrated by 12 weeks treatment with sildenafil (20 mg/three times per day) in patients with idiopathic pulmonary fibrosis (IPF).[57,58] These studies documented the absence of a direct relaxant vascular effect, while an anti-contractile and anti-remodeling effect of sildenafil explaining its beneficial effects. The same mechanisms are proposed as the rationale for using inhaled NO for interstitial lung disease caused by COVID-19.[57]

Abnormal clotting and thrombosis in COVID-19 patients significantly affect the incidence of complications and are one of the most important variables associated with mortality so much so that the prevention of venous thromboembolism in SARS-CoV-2 subjects has been recommended by the World Health Organization. Again, this evidence supports the use of sildenafil in COVID-19 infected patients, as its role in inhibiting neointimal formation and platelet aggregation via the NO/cGMP/PKG pathway is well recognized.[59] Pro-thrombotic clotting abnormalities are more evident in diabetics, and literature data suggest that the positive modulation by sildenafil of cytokines, inflammatory, and endothelial markers may have a beneficial effect on clotting in such patients.[60]

We performed a systematic review to identify ongoing trials of COVID-19 patients that target the NO-cGMP-PDE5 axis, finding 6 out of 1717 registered studies (https://clinicaltrials.gov).

We performed a web search on https://clinicaltrials.gov/ with COVID as fixed "condition or disease" search term and "sildenafil,"

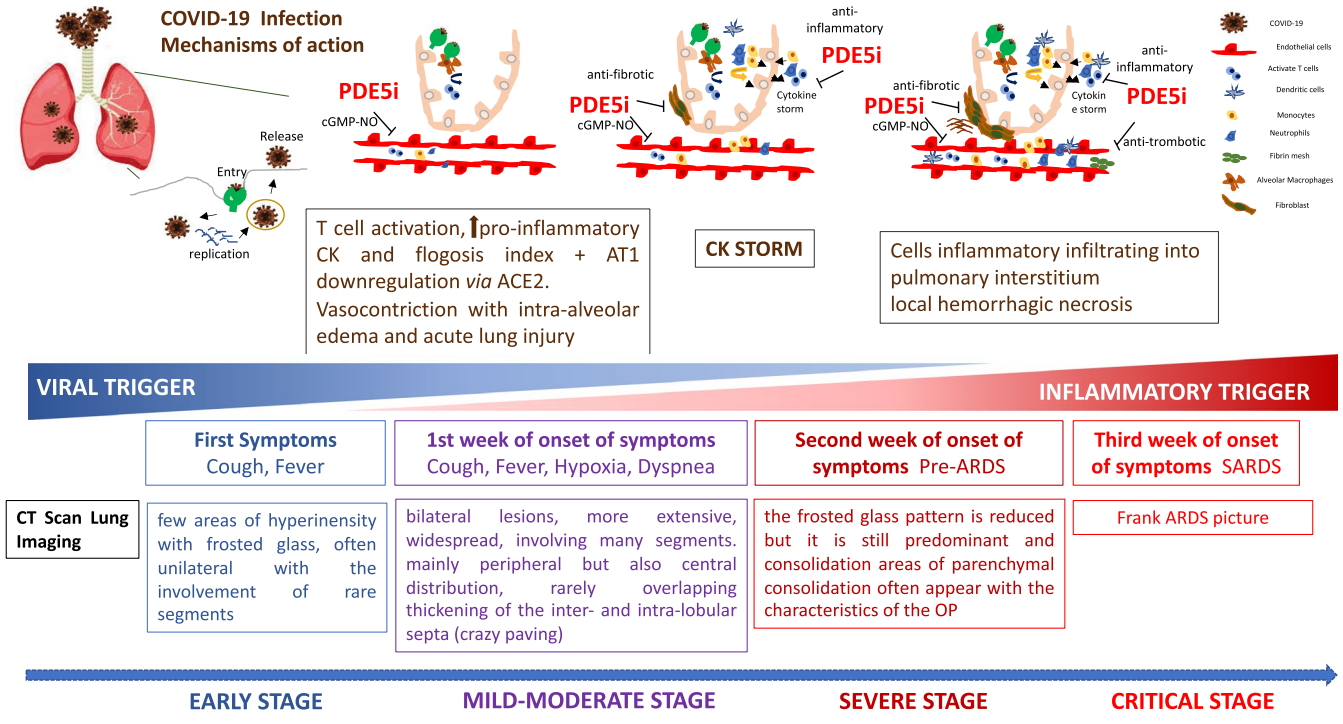

FIGURE 1 Proposal for stage-specific PDE5i therapy in counteraction of COVID-19 mechanisms of damage

"tadalafil," "vardenafil," "avanafil," "PDE5 inhibitor," "phosphodiesterase inhibitor" alternatively used as other terms.

Five of these focus on different NO inhalation therapy regimens, respectively, exploring: (a) inhaled NO (140-300 ppm for 20-30 minutes) versus placebo in 260 subjects (recruitment ended) to prevent the deterioration of mild COVID-19 infection (NCT04338828); (b) inhaled NO (140 - 180 ppm for 20-30 minutes for 14 days) versus control in 240 subjects (recruiting) to prevent deterioration from mild to severe COVID-19 infection (NCT04305457); (c) inhaled NO (160 ppm for 15 minutes) versus control in 470 healthcare professionals dedicated to care for patients with proven SARS-CoV-2 infection (not yet recruiting) to estimate the percentage of subjects with a positive test in the two groups (NCT04312243); (d) inhaled NO (concentration 80 ppm for 48 hours, followed by 40 ppm, followed by weaning before stop) versus control in 200 subjects (recruiting) to measure changes in arterial oxygenation at 48 hours from enrollment (NCT04306393); (e) inhaled NO (160 ppm for 26 days) in 20 subjects (recruiting) to measure the safety of enrolled patients (NCT03331445): Interestingly, this trial, which explores a new target therapy for Corona-like virus lung infections, was first posted on November 6, 2017. The final trial (NCT04304313) is a pilot study exploring the effects of sildenafil 0.1 g/day for 14 days in 10 subjects, with the rate of disease remission as the primary outcome. Table S1 summarizes these studies.

Given these evidences, we believe that the administration of sildenafil, as an adjunct to the current standard protocols, to hospitalized men with metabolic syndrome and/or T2DM who have confirmed SARS-CoV-2 infection and mild-to-severe symptoms of COVID-19 may offer a therapeutic benefit by targeting inflammation and fibrosis, and hence reducing the progression of lung disease and associated systemic complications. Our hypothesis is that sildenafil could act by: (a) counteracting the Ang-II-mediated downregulation of AT-1 receptor (initial first phase); (b) acting on monocyte switching, thus reducing pro-inflammatory cytokines, interstitial infiltration and the vessel damage responsible for alveolar hemorrhage-necrosis; (c) inhibiting the transition of endothelial and smooth muscle cells to mesenchymal cells in the pulmonary artery by inhibition of extracellular kinase 1 and 2 (ERK1/2) and SMAD3 phosphorylation; (d) inhibiting intrapulmonary vasoconstriction caused by AT1 receptor downregulation due to SARS-CoV-2-ACE2 binding alveolar cells, bronchial epithelium, and vascular endothelium,[9,61] see Figure 1. This hypothesis has been formalized in the Italian DEDALO project, whose authorization by the Italian Ministry of Health is pending.

The safety profile of long-term PDE5 inhibitor use has been extensively investigated,[31,62,63] including in relation to antiretroviral drugs in HIV patients.[64-69]

It is this context, in which we apply in late April at call COVID-19 from the Italian Ministry of Health with our propose: "SilDEnafil administration in DiAbetic and dysmetabolic maLe patients with COVID-19. The DEDALO trial" submitted as a phase 3 multicenter randomized, interventional, controlled trial. This trial aims to evaluate the effect of oral sildenafil on the rate of disease remission (defined as improvement in symptoms or lung imaging and/or SPO2 > 93% or PaO2/FiO2 > 300 mmHg without oxygen inhalation) in *mild-moderate patients*, while in *severe patients*, rate of entering mechanical ventilation. Time for hospitalization, stabilization or increase of the P/F (over 15%), reduction in PEEP absolute value (over 20%), rate of symptoms, inflammatory/endothelial function markers and CKs or lung imaging recovery, rate of undetectable viral RNA (continuous twice), adverse events, infectious susceptibility, and the long-term effect on lung damage will also detected.

The overall enrolment will include 100 Italian hospitalized diabetic and dysmetabolic men (at high risk) diagnosed with mild to moderate and severe COVID-19 infection. They will be randomized to sildenafil citrate 60 mg (20 mg oral tablets, 1 pill TID) versus control for 8 weeks.

If the findings of the various ongoing clinical trials are positive, the low cost, wide availability, and temperature stability of PDE5 inhibitors could make them a major resource to combat COVID-19, especially in developing countries.

2 | CONCLUSIONS

There is strong evidence that PDE5 inhibitors could modulate the harmful effects resulting from over-stimulation of the immune system, opening up a new scenario for their use in COVID-19 patients. Given the devastating economic consequences of COVID-19 on national health systems worldwide and the costs of the intensive care units needed to manage critical patients, the use of PDE5 inhibitors may offer a cheap, readily available and non-experimental treatment strategy to stop the disease from progressing to its most severe final stages, in which current treatments are unfortunately not always effective.

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CONFLICT OF INTEREST

Nothing.

AUTHORS' CONTRIBUTIONS

AMI, EG, and RP conceived and designed the opinion. MAV, FC, and DG collected the data. AMI, EG, and RP co-wrote the manuscript. CMM, AI, and Gd'E contributed to the revision of the manuscript. All authors have read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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