ABSTRACT COVID-19 pandemic is responsible for near to two million deaths worldwide. Because of the lack of any specific treatment option, repurposing of existing drugs continues worldwide to find immediate therapeutic strategies for deadly SARS-CoV-2 infection. We recently demonstrated the efficacy of Dobesilate, an old medicament with a long history of use with proven efficacy and good safety profiles, in a patient suffering from COVID-19 disease. The postulated therapeutic mechanism of Dobesilate in COVID-19 disease may primarily involve the following effects: reducing infection-related pathways, suppressing inflammation-related pathways (cytokine storm), protecting capillary barrier permeability, alleviating thrombosis and perivascular oedema, and ultimately attenuating interstitial fibrosis.

KEYWORDS SARS-CoV-2, COVID-19, Dobesilate, FGF, VEGF

Introduction

The coronavirus (COV), now named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is responsible for the coronavirus disease 2019 (COVID-19). It was first detected in early December in Wuhan City, Hubei Province, China. The disease was initially described as “pneumonia of unknown etiology” with a high fever that was not responding to drug treatment. Approximately 80% of people infected with COVID-19 present with mild to moderate symptoms that may include pneumonia, most of whom recover spontaneously. Some patients (14%) have severe symptoms with blood oxygen saturation (<93%), and 6% are critical with respiratory failure, septic shock and/or serious multi-organ dysfunction or failure [1]. COVID-19 is a new disease that rapidly became a dominant global health issue. Our current understanding of its pathogenic mechanism is very limited. However, it is evident, according to recent reports, that the disease may evolve in four overlapping phases (Figure 1): Phase I: the host-virus interactions take place, dictating the outcome for the subsequent phases of the disease. Phase II: the hyper-responsiveness of the immune system is characterized by the apparition of a “cytokine storm”. In 20% of patients, the disease may become severe and/or critical. Phase III: corresponds to a state of hypercoagulability. Finally, Phase IV: organ damage and failure occur, related to organ and cell-specific expression of angiotensin-converting enzyme 2 (ACE2) receptors, the intensity of the inflammatory response and the hyper-coagulable state [2,3]. Considering the high mortality rate of COVID-19, the development of effective therapeutics is an urgent issue and requires the identification of quality targets.

1. SARS-CoV-2 interactions with the host cells

The SARS-CoV-2 first predominantly infects airways, mainly ciliated bronchial epithelial cells and type 2 pneumocytes using the angiotensin-converting enzyme 2 (ACE2) as a receptor [4]. SARS-CoV-2 S1 protein contains a receptor to bind to ACE2. The serine-threonine protease (TMPRSS2) uses SARS-CoV-2 to prime the spike S1 protein and facilitate binding to ACE2 and entry into the cell [5,6]. Cathepsin L (CATL) plays an important role in entry into the cell by endocytosis [6-8] (Figures 2 and 3). Cathepsin L is a cysteine protease that mediates the cleavage of the S1 protein of SARS-CoV-2 surface necessary for entry into human host cells [7] that activate fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) signals pathways.
Recent data suggest that another protein/receptor termed neuroligin 1 (NRP1) is also implicated in the SARS-CoV-2 infection [9-12]. NRP-1 has been implicated in COVID-19 by enhancing the entry of SARS-CoV-2 into the brain, originating neurological manifestations in COVID-19 patients [12-14]. Due to the lack of cytosolic protein kinase domain, NRP1 acts primarily as a co-receptor for various extracellular ligands, including FGF and VEGF [11,12,15]. It thus induces a multitude of effects such as cell proliferation, angiogenesis and inflammation [10,11,12,16]. Experimental data support a cardinal role of FGF for virus entry in the host cells [17]. It has been demonstrated that FGF promotes SARS-CoV-2 replication through the interferon (IFN) alpha pathway [18] (Figure 4). Consequently, FGF has a double effect in viral infection: first, it contributes to virus entry, and secondly, it promotes viral replication in the host cell cytoplasm. These results reinforce the notion that inhibition of FGF triggers an antiviral program [19].

Figure 1. Phases of SARS-CoV-2 infection.

Figure 2. Dobesilate would interfere with the interaction of SARS-CoV-2 with the cellular ACE2 target receptor. The binding of spike protein (S-protein) of virus surface to angiotensin-converting enzyme-2 (ACE2) allows for the entry of the virus into the target cell. SARS-CoV-2 uses heparan sulfate to bind the cell and to progress on the cellular surface for reaching its cellular target receptor, ACE2 (left panel). Dobesilate (DOBE) has a high affinity for heparan sulfate. DOBE would impede viral particle binding and progression to the target receptor, inhibiting viral infective capacity (right panel).

Figure 3. Dobesilate would avoid FGF-mediated promotion of SARS-CoV-2 infection of target cells. Transmembrane serine protease 2 (TMPRSS2) activates viral spike protein (S-protein), promoting virus binding to angiotensin-converting enzyme-2 (ACE2). Internalization of ACE2-SARS-CoV-2 complex and target cell infection is promoted by cathepsin-L. Fibroblast growth factor (FGF) induces activation of TMPRSS2 and enhances cathepsin-L, thus favouring viral infection of the target cell (left superior panel and right panel). Dobesilate (DOBE) possesses demonstrated capacity as an FGF inhibitor. By inhibiting FGF activities, the presence of Dobesilate would result in inhibition of viral infection by reducing TMPRSS2 activation and cathepsin-L upregulation (left inferior panel) and avoiding internalization of the viral particles into the target cell.

2.Molecular pathways implicated in phases II, III and IV of COVID-19 disease

Several molecular overlapped hyperactive signals have been reported in COVID-19 disease. These signals produce histopathological alterations, first in the lung and secondly in the organs with ACE2 receptors. Besides lung pathology, tissue disturbance has been observed in the liver, kidneys, intestines, brain, heart and blood vessels [20]. Below, we describe the most important up-regulated signals and molecules which play a cardinal role in the progression of COVID-19.

2.1.Hyperinflammation

SARS-CoV-2 is a potent inducer of inflammatory cytokines during COVID-19. The “cytokine storm” or “cytokine cascade” is originated by a virus which activates immune cells and induces the secretion of several inflammatory cytokines (IFNδ, TNFa, IL-1β, IL-8, IL-12, IL-16, IL-18, IL-33 and TGFβ) and chemokines (CCL2, CCL3, CXCL8, CXCL9, CXCL10) [21,22]. “Cytokine storm” is the postulated mechanism for organ damage and is a major contributor to the high mortality rate. Moreover, it also may lead to heart attacks and strokes in COVID-19 patients [20]. As well, nuclear factor Kappa-b (NF-Kb) and signal transducer activator and transcription 3 (STAT3) pathways hyper-activation play a pivotal role in SARS-CoV-2 infection [23-25].
It has been postulated that COVID-19 is a vascular illness. The systemic leakiness and adhesiveness of the deregulated vascular endothelium might play a central role in the pathogenesis of COVID-19 disease [5, 26-28]. The endothelium, which forms the inner cell lining of all blood and lymphatic vessels in the body, is a spatially distributed organ. In adult people, endothelium weighs approximately 1 kg and covers a total surface area of 4000 to 7000 square meters. The endothelium is involved in most, if not all, disease states, either as a primary determinant of pathophysiology or as a victim of collateral damage. The vascular endothelium is an active paracrine, endocrine and autocrine organ that is indispensable for the regulation of vascular tone and maintenance of vascular homeostasis [29]. Inflammation may lead to endothelial dysfunction, which can induce a procoagulant state [30]. It has been proposed that alveolar viral damage may trigger an underlying inflammatory reaction, promoting a pulmonary microvascular thrombosis or endothelial thrombo-inflammatory syndrome, affecting microvascular beds beyond the lung (e.g. heart, kidney, brain and other vital organs) [31].

Up-regulation of pro-inflammatory cytokines, proteases, leukotrienes, platelet-activating factor (PAF), and oxidants produced by the neutrophils [32] culminate in the destruction of the lung glyocalyx. This process may increase the permeability of the endothelium, allowing the viruses to pass to the bloodstream and reach other organs that express ACE2. Endothelial hyperpermeability also promotes infiltration of neutrophils and inflammatory monocytes in the pericapillary space. Increased permeability of microvascular barriers also result in protein-rich plasma fluid accumulation in air spaces and decrease blood levels [33]. When vascular permeability and systemic inflammation are too high, vascular dysfunction and inflammation lead to multiple organ failure [34].

Also, nitric oxide (NO) plays an important role in endothelium physiology [35]. Recently it has been postulated that NO has a crucial role in COVID-19 disease [36]; indeed, restoring NO may attenuate endotheliitis and contribute to pulmonary vasodilation, antithrombotic and direct antiviral activities [37]. Additionally, NO interferes with the interaction between coronavirus S1 protein and ACE2 receptor. NO inhibits the replication cycle of severe acute respiratory syndrome coronavirus [38]. Furthermore, TMPRSS2, critical in viral cellular entry, appears to be NO sensitive [39]. Endothelial inflammation (endotheliitis) in COVID-19 patients may occur through multiple mechanisms. This includes cytokine storm, the activation of the complement components in blood neutrophils extracellular traps (NETs), or a direct result of SARS-CoV-2 infection of endothelial cells through the ACE2 receptor [40].

2.2. Endotheliitis

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2.7. Fibrosis

Fibrosis is the final stage in many inflammatory diseases and is defined as an abnormal accumulation of extracellular matrix (ECM) components. Fibrosis can affect many tissues, and numerous studies have been conducted to find satisfactory treatments. Interstitial pulmonary fibrosis can develop following inflammation in COVID-19 subjects [55-57].

2.8. Sepsis

Sepsis is a pathological host response to infection leading to vascular barrier breakdown due to elevated level of FGF and VEGF [58]. It has been reported that inhibition of VEGF improves experimental sepsis morbidity and mortality, making it a potential therapeutic against sepsis condition [59]. Sepsis is one fatal complication of COVID-19 evolution that correlates with FGF and VEGF up-regulation [60]. Thus, inhibition of FGF and VEGF signalling pathways could represent an attractive therapy for sepsis in COVID-19 pathophysiology.

3. The roles of FGF and VEGF in the pathophysiology of COVID-19 disease

Fibroblast growth factor (FGF) consists of 18 members of secreted proteins critical in controlling cell proliferation, inflammation, angiogenesis and organ repair and regeneration [61]. FGF signals are mediated via the activation of a set of cell surface receptors (FGFRs). FGFRs are single-pass transmembrane proteins with tyrosine kinase activity, and FGF-FGFRs interactions are stabilized by heparan sulphate proteoglycans at the cell surface [62]. FGF production is tightly regulated in normal conditions by its sequestration in the cellular matrix (ECM) because it controls several physiological functions [63,64]. In some pathological conditions, proteases liberate ECM-bounded FGF, which participates in the pathophysiology of inflammation and angiogenesis-dependent diseases. Recently, it has been reported that FGF has a key role in viral infection [65,66], suggesting that inhibition of FGF signals represent an attractive new antiviral therapy.

While vascular endothelial growth factor (VEGF) mainly targets endothelial cells, it has been shown that this factor has multiple effects on other cell types. Although there are several related genes, including VEGF-B, VEGF-C, VEGF-D and placental growth factor (PLGF), most attention is focused on VEGF-A due to its key role in regulating angiogenesis and in its essential role in diverse cells and tissues physiologic homeostasis. VEGF and its isoforms have a differential ability to bind heparin [29]. VEGF-A165 is the most physiologically relevant isofrom with a single heparin-bind domain that, in part, is sequestered in the extracellular matrix (ECM) [29]. Experimental and clinical data suggested that both FGF and VEGF have an important role in activating signal pathways in COVID-19 pathophysiology [67-73].

Patients with fragile already impaired endothelium and in particular with chronic comorbidities (such as obesity, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, chronic respiratory disease, malignancy) are at particular risk of severe (defined as a severe disease based on clinical symptoms, ICU (intensive care unit) admission, and death) or fatal COVID-19 disease [74-76]. Indeed, SARS-CoV-2 infection in such patients may exacerbate the underlying endothelial dysfunction [77]. In a French multicenter observational study in people with diabetes hospitalized for COVID-19 (Coronado study), age, microvascular and macrovascular diabetic complications, treated obstructive sleep apnoea, dyspnoea and some biological variables (i.e. increased AST and CRP and decreased eGFR and platelet count on admission) were independently associated with the risk of early death [78].

4. Dobesilate

Intensive research from our team conducted for the synthesis of FGF inhibitors as well as in the screening of old medicaments with pathologic FGF inhibitor activity concluded that the most potent FGF inhibitor was Dobesilate (2,5-dihydroxyphenyl sulfonate). Dobesilate is the most active member of a family of compounds headed by gentisic acid, the catabolite of aspirin, which inhibit FGF and VEGF-A165 induced proliferation, migration and angiogenesis, in distinct biological scenarios, includ-
ing inflammation and angiogenesis-dependent diseases [79-82]. Calcium dobesilate (CaD, Doxium®) [83] is a small synthetic molecule with a good safety profile that has been widely used for more than six decades as a vasoactive and angioprotective drug improving endothelial dysfunction [84-85], to treat multiple diseases, such as microangiopathies (in particular diabetic retinopathy), chronic venous insufficiency and haemorrhoidal disease [86-89]. The principal advantage of Dobesilate is to reduce FGf and VEGF-A165 exaggerated signalling while maintaining the physiological effects of both growth factors and consequently avoiding adverse effects [90]. In several human diseases, Dobesilate has shown clinical efficacy [91-93]. Recently Dobesilate demonstrated demonstrated efficacy in a single COVID-19 patient [66]. Figure 5 shows the capacity of Dobesilate to inhibit several, if not all, signalling pathways implicated in COVID-19 pathophysiology, supporting the use of Dobesilate as a new possible therapy that should be effective in COVID-19 patients.

Conclusion

Up to date, no approved drugs are targeting specifically SARS-CoV-2. Until a treatment is available, repurposing approved drugs could significantly shorten the time and reduce costs compared to “de novo” discovery. The simultaneous inhibition of multiple targets is expected to have higher therapeutic potential than single-target approaches to prevent COVID-19 disease progression. According to its pharmacological activities, Dobesilate is a potential new therapeutic against FGf and VEGF signalling pathways which have a key role in COVID-19 pathophysiology. Due to its long experience on the market, its well-tolerated safety profile and convenient posology (1-2 g/d p.o.) [83,86], as well as the two-axis rationale (Fig. 6A) to dampen both viral infectivity and endothelial inflammation / dysfunction, Dobesilate could represent an interesting alternative (Fig. 6B) for the treatment of Covid-19 disease.

Author’s Contributions

PC wrote the article, JA and DZ draw the graphs, AM, JLC and GGG read the article and gave their approval.

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Conflict of interest

There are no conflicts of interest to declare by any of the authors of this study.

References


73. White D, Stephen MacDonald, Tara Edwards, Chris Bridge-


83. Swiss SmPC: https://compendium.ch/product/19768-doxium-caps-500-mg/mpro.


