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feature

Taming the cytokine storm: repurposing montelukast for the attenuation and prophylaxis of severe COVID-19 symptoms

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As a result of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, a clinical complication can arise that is characterized by a hyperinflammatory cytokine profile, often termed a 'cytokine storm'. A protein complex (nuclear factor kappa-light-chain-enhancer of activated B cells; NF-κB) is intricately involved in regulating inflammation and the immune response following viral infections, with a reduction in cytokine production often observed following a decrease in NF-κB activity. An approved asthma drug, montelukast, has been found to modulate the activity of NF-κB, and result in a corresponding decrease in proinflammatory mediators. Herein, we hypothesize that repurposing montelukast to suppress NF-κB activation will result in an attenuation of proinflammatory mediators and a decrease in cytokine production, thereby leading to a reduction in symptom severity and to improved clinical outcomes in patients with Coronavirus 2019 (COVID-19).

Introduction

The current COVID-19 pandemic has developed into one of the most significant health issues of modern times, while simultaneously inflicting a considerable economic toll. Effective therapies are urgently required to mitigate the humanitarian cost of SARS-CoV infections, and provide for improved clinical outcomes of patients. The development of acute respiratory distress syndrome (ARDS) and cytokine storm syndrome (a hyperinflammatory cytokine profile) are two of the main complications observed in patients with COVID-19, with NF-κB serving as a key gate keeper in controlling the cellular responses to viral infections. NF-κB is a protein complex involved in adaptive and innate immune responses, inflammation, and the production of cytokines. We propose that targeting suppression of NF-κB activation in patients with COVID-19 will result in a corresponding decrease in proinflammatory mediators and culminate in a reduction of cytokines, thereby reducing the severity of COVID-19 symptoms and lead to improved clinical outcomes.

To rapidly identify a suitable COVID-19 drug, a repurposed drug approach (using a known drug for an alternate indication) would provide for the quickest pathway to identify a safe and effective therapy. Montelukast is an approved generic asthma drug that has been shown to inhibit the signaling of NF-κB and downstream proinflammatory mediators, including interleukins (IL) 6, 8, and 10, tumor necrosis factor (TNF)-α, and monocyte chemo attractant protein 1 (MCP1) (among others). Herein, we outline the scientific rationale for repurposing montelukast to manage the symptom severity of SARS-CoV-2 infections, via modulation of the NF-κB pathway, and a corresponding decrease in cytokine production.

Figure 1 briefly details the role of leukotrienes in activating the NF-κB pathway, leading to the increased expression of proinflammatory mediators. Cellular SARS-CoV-2 infection/replication (inflammatory stimulation) leads to a

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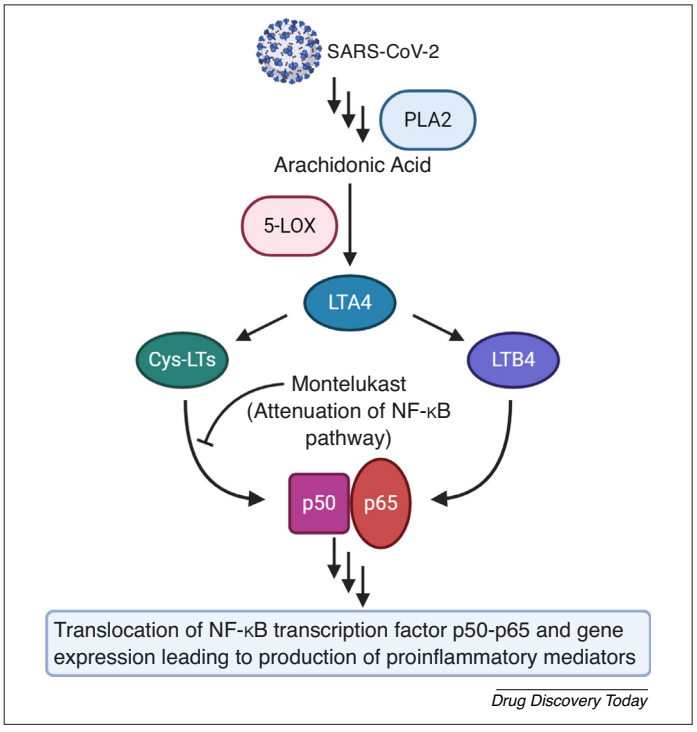

FIGURE 1

Antagonism of cysteinyl leukotriene receptor 1 by montelukast attenuates the activation of nuclear factor (NF)-κB transcription factor p50-p65, decreasing downstream gene expression and production of proinflammatory mediators, and mitigating cytokine storm syndrome. Abbreviations: 5-LOX, 5-lipoxygenase; Cys-LTs, cysteinyl leukotrienes; LTA4, leukotriene A4; LTB4, leukotriene B4; PLA2, phospholipase A2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

corresponding release of arachidonic acid via phospholipase A2 (PLA2) within the cell, and subsequent conversion into leukotriene A4 (LTA4) via 5-lipoxygenase (5-LOX). LTA4 leads to the production of leukotriene B4 (LTB4) and the cysteinyl leukotrienes (Cys-LTs; LTC4, LTD4, and LTE4), which bind to their respective leukotriene receptors and activate the NF-κB pathway, via NF-κB transcription factor (TF) p50-p65, leading to the downstream production of proinflammatory mediators [1]. Montelukast is a selective cysteinyl leukotriene receptor 1 antagonist that blocks the binding of the cysteinyl leukotrienes, attenuating activation of the NF-κB pathway and leading to a corresponding decrease in the production of proinflammatory mediators. We propose that repurposing montelukast to treat patients with COVID-19 could alleviate some of the severe symptoms that lead to ARDS and cytokine storm syndrome, via modulation of the NF-κB pathway and a decrease in cytokine production.

Herein, we present a scientific basis for using montelukast to manage the symptoms of COVID-19, which we believe warrants rapid and thorough evaluation by the clinical community, primarily because of the low risk and high safety profile of montelukast, and the potential to alleviate cytokine storm syndrome, thereby reducing the prevalence of severe COVID-19 symptoms.

ARDS and cytokine storm

ARDS, which ultimately leads to life-threatening respiratory failure, is the major complication of patients hospitalized with COVID-19. Efforts are being made to understand the pathophysiology of this disease and, currently, several clinical reports have demonstrated that the disease is associated with mild and severe forms of cytokine storm syndrome in critically ill patients and, hence, is thought to be related to the major cause of death [2,3]. The major reported inflammatory cytokines found in plasma of clinically ill patients with COVID-19 are: IL-2, -6, -7, and 10; granulocyte-colony stimulating factor (G-CSF); interferon-γ-inducible protein 10 (IP10); MCP1; macrophage inflammatory protein 1 alpha (MIP1A); and TNF-α. This hyperinflammatory cytokine profile is particularly consequential for a subpopulation of severely ill patients [2,3] with the release of IL-6 from these cascades (cytokine storm) resulting in worse clinical outcomes for patients in intensive care units (ICU) [3]. These reports from various investigators in patients with confirmed COVID-19 suggest the role of cytokine inflammatory mediators in the disease pathology of critically ill patients. As a result, the development of new therapeutics that attenuate both ARDS and/or cytokine storm syndrome are urgently required to treat patients with severe COVID-19, because there are currently no approved therapies to treat SARS-CoV-2 infections.

Clinical role of NF-κB inflammatory mediators in COVID-19 pathogenesis: the NF-κB butterfly effect

NF-κB is a ubiquitous TF that is associated with inflammatory and immune responses [4] and is associated with a 'cytokine storm'. NF-κB is the first crucial factor in many inflammatory responses and results in a downstream cytokine release/cascade. RNA viral pathogens are known to activate NF-κB and its corresponding cytokine cascade [5], thereby acting as a malevolent activator of the NF-κB pathway [6]. Therefore, an initial inflammatory response from viral infections is often characterized by the production of NF-κB. In-addition, activation of NF-κB can yield a better environment for replication of the virus as a means to adapt to the host immune system, with the attenuation of the NF-κB signaling pathway proposed as a novel concept for antiviral therapy [7]. Since the 2002–2003 SARS epidemic, several studies have shown that coronaviruses (MERS-CoV and SARS-CoV) activate the NF-κB pathway, which suggests that SARS-CoV-2 also activates the NF-κB signaling pathway [8], and serves as the impetus of the COVID-19 cytokine storm, loosely referred to here as the 'NF-κB butterfly effect'.

NF-κB and COVID-19 severity: sex, age, obesity and ACE2

Sex and gender differences have been observed as having an impact on the severity of symptoms and mortality of patients with COVID-19, with males being disproportionately affected [9]. A recent study from patients in New York City found that just over 60% of patients hospitalized with COVID-19 were men, with mortality higher among men at almost all age intervals [10]. It has been proposed that sex hormones produced by females help to defend against coronaviruses, with researchers testing estrogen for its ability to reduce the severity of COVID-19 symptoms [11]. Interestingly, estrogen (17β-estradiol) has long been known to modulate NF-κB, downregulating cytokine production and NF-κB activation, and inhibiting inflammatory responses [12,13]. The effects of estradiol on NF-κB, and the lower mortality rates of females, insinuate that suppression of NF-κB activation has a key

role in attenuating the severity of COVID-19 symptoms. This scientific precedence gives credence to our premise that small NF-κB-modulating molecules, such as montelukast, could suppress proinflammatory mediators and cytokine production, alleviating symptoms associated with cytokine storm syndrome.

Age is one of the major risk factors associated with disease severity for COVID-19, with individuals older than 65 years of age having higher mortality rates [10]. The NF-κB signaling pathway is proposed to have a key role in aging and NF-κB activation is elevated in a variety of tissues with aging. NF-κB transcriptional activity is also increased in numerous age-related degenerative diseases, such as Alzheimer's, diabetes, and osteoporosis [14]. During aging, adaptive immunity declines, whereas innate immunity becomes activated, inducing a characteristic proinflammatory profile regulated by the NF-κB pathway [15]. As a result, SARS-CoV-2 infections might exacerbate this activated proinflammatory profile of older patients, resulting in higher levels of proinflammatory cytokines and leading to poorer clinical outcomes.

In several studies, obesity has been strongly associated with worse outcomes for patients with COVID-19 and is a major risk factor for disease severity. Obesity, defined as a body mass index (BMI) >30 kg/m^2, is associated with a significant increase in risk for ICU admission, even after adjustment for age [16]. Morbid obesity (BMI > 40 kg/m^2) is independently associated with mortality for patients younger than 50 [17]. Furthermore, additional studies have highlighted the association of obesity with disease severity in patients with COVID-19 [18], particularly in younger patients [19]. Interestingly, NF-κB is crucially involved in the development of the low-grade chronic inflammation state that is associated with obesity-induced metabolic diseases and NF-κB activation further enhances the proinflammatory profile associated with obesity [20]. Overall, the NF-κB pathway is known to have a key role in the origins and perpetuation of the inflammatory state that underlies obesity-induced metabolic diseases [21]. Recently, it was shown that suppression of the IKK/NF-κB pathway, via macrophage major vault protein, constrains metabolic inflammation, and attenuates obesity-associated metabolic disorders. As described here, this link between obesity, COVID-19 disease severity, inflammation, and NF-κB activation adds further support to our premise that modulation of the NF-κB pathway could serve as a useful therapeutic target for reduction of COVID-19 disease severity and cytokine storm syndrome.

In parallel, current studies have shown that angiotensin converting enzyme 2 (ACE2) is used as the host cell entry receptor by the COVID-19 virus [22]. Thus, it has been hypothesized that ACE2 dysregulation has a crucial role in the pathophysiology of COVID-19 and leads to acute lung injury (ALI) and ARDS. It has been speculated that virus binding to ACE2 attenuates residual ACE2 activity, further skewing the ACE/ACE2 balance to a state of predominant ACE/AngII/AT1 axis signaling [23]. Furthermore, previous ground-breaking studies showed the link between cardiovascular system ACE2 and NF-κB [24], confirming the role of Angiotensin II in activating NF-κB through the AT1 and AT2 receptors [25]. As a result, modulation of NF-κB might negate the effects of SARS-CoV-2 on ACE2 dysregulation, through deactivation of NF-κB, mitigating ALI and ARDS.

Montelukast

Montelukast is highly selective cysteinyl leukotriene receptor antagonist that was approved by the US Food and Drug Administration (FDA) and Health Canada in 1998. It is extensively used orally in different forms; film-coated tablet, chewable tablets, and oral granules for maintenance treatment of chronic asthma and the prevention of exercise-induced bronchoconstriction. It is also widely approved for the relief of symptoms of both seasonal and perennial allergic rhinitis. Montelukast has an excellent safety profile and is approved for pediatric patients as young as 2 years of age and studied in patients as young as 6 months. A typical dose of montelukast is 10 mg/day, although studies administering 200 mg/day in adult patients for 22 weeks, and 900 mg/day in short-term studies (1 week) observed no clinically important adverse experiences [26]. Montelukast is highly selective against leukotriene receptors that bind C4, D4, and E4 leukotrienes (antagonism), thereby exerting anti-inflammatory effects, including: reducing airway edema, diminishing smooth muscle contraction (bronchoconstriction), downregulation of human monocyte chemotaxis induced by MCP-1 [27], and reduced inflammation.

After its development as a drug for patients with asthma, further research explored its potential in attenuating inflammatory mediators. Research showed that montelukast inhibited NF-κB signaling in a dose-dependent manner in a human acute monocytic leukemia cell line (THP-1), indicating that it might have an effect in controlling the cascade of cytokine release. Montelukast has also been shown to inhibit lipopolysaccharide-induced IL-6, TNF-α, and MCP-1 production in the peripheral blood mononuclear cells of control patients and patients with asthma. These findings demonstrated that high doses ($>10^{-6}$ M) of montelukast modulated the production of IL-6, TNF-α, and MCP-1 through the inhibition of NF-κB activation [28]. These results have also been supported by other groups, who have shown that montelukast attenuates TNF-α-mediated IL-8 expression in a dose-dependent manner by inhibiting NF-κB in a human macrophage cell line [29]. Another study demonstrated that montelukast halted the production of IL-10 and also inhibited the production of NF-κB in guinea pigs with asthma [30]. Recent studies showed that low dose combination of montelukast and levocetirizine, an antihistaminic drug, was effective in inhibiting various inflammatory mediators (the NF-κB pathway) in the respiratory biochemical cascades that are utilized by RNA viruses during infection (Ebola and Dengue) [31]. Additional results showed a decrease in viral load, via control of proinflammatory mediators through modulation of NF-κB.

Concluding remarks and target populations for study

Taking all of these points into consideration, we can conclude that transcriptional factors, such as NF-κB, could have a key role in producing the cytokine storm and initiating the surge in production of proinflammatory cytokines. As is evident by the current clinical manifestations of COVID-19, severe lung injury following SARS-CoV-2 infection is often observed, leading to life-threatening pneumonia and ARDS. Suppression of NF-κB could help to attenuate proinflammatory cytokine release and tame the cytokine storm by dampening the NF-κB butterfly effect. Therefore, we propose that it is possible to reduce the progression and severity of COVID-19-related pneumonia/ARDS through the modulation of NF-κB activation, and a subsequent reduction in the release of proinflammatory cytokines via montelukast. Given that NF-κB might serve as an attractive COVID-19 drug target, utilizing montelukast as a therapy, either prophylactically and/or post-infection, could attenuate clinical symptoms caused by COVID-19, and reduce the overall severity, and rates of mortality from SARS-CoV-2 infection.

Given the rapidly developing nature and severity of the COVID-19 pandemic, we argue that the risk–benefit ratio of using montelukast is manageable when considering the excellent safety profile of this drug and the relatively few

reports of drug–drug interactions, contraindications, or reports of serious adverse effects.

Herein, we outlined the scientific basis for using montelukast to manage the symptoms of COVID-19, which we believe warrants rapid and thorough evaluation by the clinical community. Currently, as part of a larger team approach, we are preparing to initiate a clinical trial protocol for the study of the efficacy of montelukast in patients who are newly identified as COVID-19 positive, The COvid-19 Symptom MOntelukast Trial (COSMO), NCT04389411. Here, we summarize different populations that we believe would benefit from montelukast, with the hope that other researchers can explore this therapeutic avenue, and assist with the safety and efficacy evaluation of montelukast in patients with COVID-19. We propose that the use of: (i) high-dose montelukast in patients with severe COVID-19, with or without other drugs, could reduce the patients' hyperinflammatory cytokine profile, leading to improved clinical outcomes; (ii) low-dose montelukast (placebo-controlled, double-blind) in patients with confirmed COVID-19 who initially exhibit mild/moderate symptoms could show a reduction in the risks of developing severe COVID-19 symptoms; (iii) low-dose montelukast, prophylactically and post infection, in high-risk front-line healthcare workers who test positive for COVID-19 could show a reduction in the risks of developing severe COVID-19 symptoms; and (iv) low-dose montelukast, prophylactically and post infection, in a cohort of vulnerable patients, such as long-term care residents, could show a reduced risk of developing severe COVID-19 symptoms, and reduced rates of mortality. As of June 4, 2020, a search of the ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform for similar studies using montelukast did not provide additional results, and highlights the novelty of this approach and the need to rapidly evaluate montelukast as a potential therapeutic for the attenuation of severe COVID-19 symptoms.

Disclaimer

The authors of this manuscript are not members of any regulated/licensed medical profession. The opinions expressed in this manuscript are not intended to be a substitute for professional medical advice.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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