Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2022**; 27(5): 166
https://doi.org/10.31083/j.fbl2705166

Review

Immunomodulatory and Anti-Inflammatory Effects of Berberine in Lung Tissue and its Potential Application in Prophylaxis and Treatment of COVID-19

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Academic Editor: Amedeo Amedei
Submitted: 24 March 2022 Revised: 5 May 2022 Accepted: 9 May 2022 Published: 20 May 2022

Abstract

Natural products with known safety profiles are a promising source for the discovery of new drug leads. Berberine presents an example of one such phytochemical that has been extensively studied for its anti-inflammatory and immunomodulatory properties against myriads of diseases, ranging from respiratory disorders to viral infections. A growing body of research supports the pluripotent therapeutic role berberine may play against the dreaded disease COVID-19. The exact pathophysiological features of COVID-19 are yet to be elucidated. However, compelling evidence suggests inflammation and immune dysregulations as major features of this disease. Being a potent immunomodulatory and anti-inflammatory agent, berberine may prove to be useful for the prevention and treatment of COVID-19. This review aims to revisit the pharmacological anti-inflammatory and immunomodulatory benefits of berberine on a multitude of respiratory infections, which like COVID-19, are known to adversely affect the airways and lungs. We speculate that berberine may help alleviate COVID-19 via preventing cytokine storm, restoring Th1/Th2 balance, and enhancing cell-mediated immunity. Furthermore, the role this promising phytochemical plays on other important inflammatory mediators involved in respiratory disorders will be underscored. We further highlight the role of berberine against COVID-19 by underscoring direct evidence from *in silico*, *in vitro*, and *in vivo* studies suggesting the inhibitory potential berberine may play against three critical SARS-CoV-2 targets, namely main protease, spike protein, and angiotensin-converting enzyme 2 receptor. Further preclinical and clinical trials are certainly required to further substantiate the efficacy and potency of berberine against COVID-19 in humans.

Keywords: berberine; COVID-19; SARS-CoV-2; anti-inflammatory; immunomodulatory; cytokine storm; Th1/Th2 balance

1. Introduction

Coronaviruses (CoVs) are enveloped viruses containing non-segmented, positive-stranded RNA genomes [1]. These viruses are known to cause a broad spectrum of diseases, including bronchitis, gastroenteritis, systemic disease, and even death in both animals and humans [2]. CoVs were identified as the causative agent of the Middle-East respiratory syndrome (MERS-CoV) and the severe acute respiratory syndrome (SARS-CoV) outbreaks, which occurred in Saudi Arabia in 2012 and in China in 2002, respectively. Recently, another CoV outbreak, namely severe acute respiratory syndrome 2 (SARS-CoV-2), the causative agent for the novel 2019 coronavirus disease (COVID-19), was first identified in Wuhan, China in December 2019 and is still ongoing, resulting in many fatalities worldwide. As of August 2021, the total number of confirmed COVID-19 cases exceeds 400 million and the total number of deaths is around 5 million worldwide [3]. Like MERS-CoV and SARS-CoV, SARS-CoV-2 is a respiratory pathogen. Most COVID-19 patients present with mild to moderate symptoms and recover without the need for hospitalization [4]. Nevertheless, severe respiratory complications are not uncommon, especially in high-risk populations including the elderly and those immunocompromised [4]. The most commonly reported clinical symptoms of COVID-19 include fever, dry cough, fatigue, myalgia, and dyspnea [5,6]. A small fraction of patients may also present with acute respiratory distress syndrome (ARDS), possibly as a result of cytokine storm development and over-exaggerated immune responses, leading to acute respiratory failure and/or multi-organ failure [7,8]. Currently, multiple vaccines are available with the promise of protection against COVID-19 [9]. Nevertheless, none of these vaccines ensure complete immunity against this dreaded disease, with many contracting COVID-19 despite being double-vaccinated [10]. Developing and maintaining strong immunity, even with vaccination, is therefore still essential, and there is ample evidence supporting the exceptional pharmacological benefits that natural products may have against myriads of viral infections [11], of which COVID-19 is no exception.

Berberine is a quaternary ammonium salt derived from the protoberberine group of benzylisoquinoline alkaloids,

with a molecular formula of $C_{20}H_{18}NO_4$and a molar mass of 336.36 g/mol [12,13]. It has been detected, isolated, and quantified from various plant families and genera including Annonaceae, Berberidaceae, Menispermaceae, Papaveraceae, Ranunculaceae, and Rutaceae [14,15]. The genus Berberis, belonging to the berberidaceae family, is well-known as the most widely distributed natural source of berberine with approximately 5% of its bark containing berberine [16]. Data from historical use and modern scientific research indicates that berberine possesses a wide range of biological and pharmacological benefits. It has been historically used in traditional Chinese medicine to treat various infectious and gastrointestinal diseases without any apparent side effects reported [17,18]. Findings from various *in vivo* and *in vitro* studies support the propensity of berberine in alleviating hypercholesterolemia [19], oxidative stress [20], inflammation [21], cardiovascular and metabolic diseases [22], and in exerting significant immunomodulatory [23], hepatoprotective [24,25], nephroprotective [26,27], and most relevant to this review, antiviral effects [28]. Considerable research suggests that berberine may prove to be useful for the prevention and/or treatment of the dreaded disease COVID-19. However, the exact molecular mechanisms underlying such effects are yet to be elucidated. This review sheds light on the potent anti-inflammatory and immunomodulatory properties of berberine in various respiratory disorders, tentatively suggesting that such effects may also be at play in the berberine-mediated effects against COVID-19. Precisely, the role of berberine in preventing cytokine storm, enhancing cell-mediated immunity, and restoring Th1/Th2 balance will be highlighted. Additionally, the role of berberine in mediating other inflammatory mediators will be discussed. Taken together, this review proposes berberine as a potent anti-inflammatory and immunomodulatory agent capable of exerting protective effects against ailments like COVID-19, characterized by immune dysregulations and elevated inflammation.

2. Effects of Berberine on Cytokine Storm Development

The reported *in vivo and in vitro* effects of berberine on cytokine storm are summarized in Table 1 (Ref. [29–33]).

Maladaptive immune responses in face of infections can result in an inflammatory response going out of control and pro-inflammatory cytokines being excessively released. Cytokine storms are associated with a wide array of infectious and non-infectious diseases and are suspected to be the main reason for mortality in many patients [34]. Multiple lines of evidence suggest the possible intervening role berberine may have against cytokine storm development in various lung pathologies. For example, in an *in vivo* study, berberine (low dose: 100 mg/kg; high dose: 200 mg/kg) was found to exert protective effects against asthma in ovalbumin (OVA)-induced male Wistar rats via suppressing the levels of the pro-inflammatory cytokines IL-6, IL-1β, and IL-17, suggesting the possible role of berberine in preventing cytokine storm in asthma [29]. In another *in vivo* study, berberine treatment (50 mg/kg) was found to be associated with a significant drop in the levels of the pro-inflammatory cytokines IL-6 and tumor necrosis factor alpha (TNFα) in chronic obstructive pulmonary disease (COPD)-induced mice, corroborating its potential protective effects against exaggerated cytokine production [30]. In another combined *in vivo* and *in vitro* study, Liang and colleagues found that berberine was capable of reducing acute lung injury (ALI) in lipopolysaccharide (LPS)-induced human bronchial epithelial cells (16HBE) [31]. Berberine (5 and 10 μM) was found to alleviate ALI in 16HBE cells via causing a significant drop in the protein and gene expression levels of the cytokines IL-6 and IL-8 in LPS-induced 16HBE [31]. Similar promising effects were observed *in vivo*, whereby a significant drop in the proinflammatory cytokine IL-6 and in the chemokine keratinocytes-derived chemokine (KC) was observed with DXM and berberine treatment (10 mg/kg) of LPS-induced C57BL/6 mice. In another *in vivo* investigation, berberine was found to ameliorate cigarette smoke (CS)-induced lung injury via reducing the levels of various cytokines [32]. In particular, berberine dampened the CS-induced rise in various cytokines and chemokines, including IL-6, TNFα, macrophage inflammatory protein 2 (MIP-2), and monocyte chemoattractant protein 1 (MCP-1) by 44%, 46%, 35%, and 27%, respectively. Interestingly, a 58% drop in myeloperoxidase (MPO) levels, a critical marker of inflammation, was also observed in CS-induced and berberine-treated mice. In accordance, in another combined *in vivo* and *in vitro* analysis, berberine was found to exert protective effects against ARDS, a deleterious outcome that develops from cytokine storm, in human umbilical vein endothelial cells (HUVECs) and in male C57BL/6 mice [33]. In particular, the therapeutic effects of berberine on ARDS were found to be mediated via attenuating damage caused to the pulmonary endothelial glycocalyx, which plays a pivotal role in regulating pulmonary barrier permeability and preventing lung injury [35]. Analysis on LPS-induced ARDS in mice found that berberine treatment (50, 100, and 200 mg/kg) markedly reduced endothelial glycocalyx degradation dose-dependently by reducing the shedding of the two main components in the glycocalyx, syndecan-1 (SDC-1) and heparan sulfate (HS) by 55% and 51%, respectively, with 200 mg/kg of berberine [35]. Consequently, berberine (50, 100, and 200 mg/kg) was found to maintain glycocalyx integrity and permeability and significantly alleviated pulmonary edema and neutrophil infiltration in LPS-induced mice. Additionally, berberine treatment (1.25, 2.5, and 5 μM) of HUVECs markedly reduced the levels of reactive oxygen species (ROS) and oxidative stress, with 5 μM of berberine resulting in an approximate 70% reduction in mitochondrial ROS, suggesting reduced inflammation. In fact, berberine treatment

Table 1. The effects of berberine on cytokine storm development.

Main effects	Experimental model	Dosage	Administration mode	Administration duration	Reference
Reduction of OVA-induced secretion of IL-4, IL-5, IL-6, IL-13, IL-17, and IL-1β levels	Male Wistar rats	100 and 200 mg/kg	Oral	28 days	[29]
Reduction of CSE-induced IL-6 and TNFα levels in BALF	C57BL/6 mice	25 mg/kg (low dose) and 50 mg/kg (high dose)	Oral	Treatment for 6 days a week for 60 days	[30]
Suppression of LPS-induced rise in IL-6 and IL-8 protein and gene expression levels in 16HBE cells	16HBE cells	2.5, 5, and 10 μM	-	Pretreatment for 4 hrs	[31]
Reduction of LPS-induced rise in IL-6 and KC levels in mice	Male C57BL/6 mice	10 mg/kg	Intraperitoneal	Pretreatment twice (at 24 and 2 hrs before LPS stimulation)	
Reduction of CS-induced rise in MPO activity Reduction of CS-induced rise in TNFα, IL-6, MIP-2, and MCP-1 levels	Male C57BL/6 mice	50 mg/kg	Intragastric	1 hr before CS exposure on 4 consecutive days	[32]
Inhibition of LPS-induced glycocalyx shedding in HUVECs and in mice	HUVEC cells	1.25, 2.5, 5 μM	-	Pre-treatment for 1 hr before LPS stimulation	[33]
Suppression of LPS-induced rise in ROS and oxidative stress in HUVECs Suppression of LPS-induced rise in IL-1β, IL-6, and TNFα levels in mice	Male C57BL/6 mice	50, 100, and 200 mg/kg	Oral	Treatment for 7 days	

(50, 100, and 200 mg/kg) of mice was found to significantly and dose-dependently attenuate the levels of the pro-inflammatory cytokines TNFα, IL-1β, and IL-6 in bronchoalveolar lavage fluid (BALF). Particularly, a 48%, 52%, and 54% drop in TNFα, IL-1β, and IL-6, respectively, was observed with the use of 100 mg/kg of berberine [35].

3. Effects of Berberine on Th1/Th2 Balance

The reported *in vivo* and *in vitro* effects of berberine on Th1/Th2 balance are summarized in Table 2 (Ref. [29,36–38]).

T-helper cells are regarded as the most prolific cytokine producers [39]. Consequently, these cells are believed to play a fundamental role in modulating immunity in different disease pathologies by directing different immune responses [40]. T-helper cells can be subdivided into type 1 helper (Th1) and type 2 helper (Th2) T cells. Th1-related cytokines are known to promote pro-inflammatory responses against intracellular pathogens whereas Th2-related cytokines are believed to play more of an anti-inflammatory role [39]. Striking a balance between Th1 and Th2 responses depending on the immune challenge in question is key to orchestrate well-coordinated immune responses to eliminate pathogens [41]. Studies suggests that an immunomodulatory agent like berberine may be capable of restoring Th1/Th2 balance in various pathological conditions, including respiratory ailments, and can help alleviate lung pathologies via increasing Th1responses and decreasing Th2 responses.

In an *in vitro* investigation, Ma and colleagues found that berberine possesses promising anti-inflammatory effects in pro-inflammatory cytokine-activated human bronchial epithelial cells (BEAS-2B) mainly via modulating Th1- and Th2-cytokine production [36]. Pro-inflammatory cytokine like IL-4 released from t helper cell type 2 (Th2) activation, can trigger the release of eotaxin-1 (CCL11), the most potent chemokine that can enhance eosinophil movement and consequently exacerbate asthma symptoms [36]. Berberine treatment (1 μM) was found to significantly suppress the levels of CCL11 and IL-6, which is also known to play a pivotal role in exacerbating asthma symptoms, in BEAS-2B cells treated with IL-4 and TNFα [36]. Berberine was found to suppress CCL11 and IL-6 levels in IL-4 and TNFα-treated BEAS-2B cells via downregulating signal transducer and activator of transcription 6 (STAT-6), but not nuclear factor kappa B (NF-κB) or p38 mitogen activated protein kinase (MAPK), signaling pathway. Further *in vivo* studies should confirm the role that STAT-6 pathway plays in regulating inflammation in asthma. Collectively, results from this study point to the promising role berberine has in suppressing exaggerated Th2 inflammatory reactions in allergic asthma. In another *in vivo* investigation, berberine was found to exert potent anti-inflammatory and immunomodulatory properties against asthma in ovalbumin (OVA)-induced male Wistar rats [29]. In particular, berberine (low dose: 100 mg/kg; high dose: 200 mg/kg) was found to result in a significant

Table 2. The effects of berberine on Th1/Th2 balance.

Main effects	Experimental model	Dosage	Administration mode	Administration duration	Reference
Reduction of IL-6 and CCL11 levels Suppression of STAT-6 pathway	BEAS-2B cells	1 μM	-	16–18 hrs	[36]
Reduction of OVA-induced secretion of IL-4, IL-5, and IL-13	Male Wistar rats	100 and 200 mg/kg	Oral	28 days	[29]
Induction of IL-12 P40 production Activation of p38 MAPK pathway	Mouse splenic macrophages	0.1, 0.5, 1 μg/mL	-	Incubation for 48 hrs	[37]
Induction of IL-12 production in mouse macrophage and dendritic cells	Mouse macrophage and dendritic cells	0.1, 0.5, 1 μg/mL	-	Pre-treatment for 6 hrs	[38]
Elevation of IFN-y levels and suppression of IL-4 levels in antigen-primed $CD4^+$ T cells Induction of IL-12 production in macrophages derived from female DBA/2 mice	Female DBA/2 mice	200 μg	Intraperitoneal	Treatment for 24 hrs	

and dose-dependent drop in the Th-2 related cytokines IL-4, IL-5, and IL-13, indicating reduced Th2 immune response overactivation.

Interestingly, in another *in vitro* analysis, berberine was found to be capable of inducing the production of IL-12, which has a role in promoting the development of Th1 immune responses via inducing IFNγ production by T and natural killer (NK) cells, in mouse splenic macrophages [37]. As such, IL-12 is believed to exert therapeutic effects against diseases with pathologic Th2 responses like asthma [42]. Further analysis from the same study found that berberine (1 μg/mL) induced IL-12 production in mouse macrophages via activating MAPK pathway rather than the major inflammatory regulatory pathway NF-κB. Future studies should therefore discern the role of p38 MAPK in suppressing inflammation and should also shed more light into the effect of IL-12 induction in asthma and COPD to confirm its therapeutic potential on such ailments. The role that berberine plays on IL-12 was also been confirmed by another combined *in vivo* and *in vitro study* by the same research team [38]. Specifically, *in vitro*, berberine (0.1, 0.5, and 1 μg/mL) was found to induce IL-12 production in antigen-presenting cells like macrophages and dendrites in a dose-dependent manner. The IL-12 enhancing effects of berberine were also apparent following induction of macrophages and dendrites with the IL-12 activators, LPS and CD40L, respectively, indicating the potent role of this natural constituent in promoting Th1 immune responses. Additionally, following the induction of IL-12, a significant increase in the Th1 cytokine IFNγ and a significant drop in the Th2 cytokine IL-4 was observed in $CD4^+$ T cells treated with an antigen that was presented using berberine-treated macrophages. *In vivo*, macrophages extracted from DBA/2 mice treated with berberine (200 μg) for 24 hrs were found to have elevated levels of IL-12, indicating enhanced Th1 immune responses. Collectively, findings from this study point to the potentially promising role berberine has in enhancing Th1 immune responses in diseases characterized by elevated Th2 responses like asthma and COPD.

4. Effects of Berberine on Cell-Mediated Immunity

The reported *in vivo* and *in vitro* effects of berberine on cell-mediated immunity are summarized in Table 3 (Ref. [29–32,43–46]).

Berberine is believed to play a critical role in strengthening adaptive immune response. Cell-mediated immunity, a type of adaptive immune response, depends largely on the function of T cells, both helper (with CD4 coreceptor) and cytotoxic (with CD8 coreceptor) [47]. Both of these cell types aid in pathogen elimination via activating other immune cells and assisting in the elimination of pathogens and other infected host cells [42]. As indicated in the previous section, berberine may be a useful agent in restoring Th1/Th2 balance in various lung pathologies. More specifically, berberine was shown to be useful for elevating Th1 immune responses in diseases characterized by elevated Th2 immune responses like COPD and asthma [48]. Because Th1 immune responses and cell-mediated immunity are two processes believed to be highly interrelated [49], berberine is expected to exert similar enhancing effects on cell-mediated immune responses.

In one *in vivo* study, berberine was found to exhibit potent anti-inflammatory and immunomodulatory properties against asthma in OVA-induced male Wistar rats [29]. In particular, berberine (low dose: 100 mg/kg; high dose: 200 mg/kg) was found to reverse the OVA-induced rise in immune cell count in rats, whereby it resulted in a significant and dose-dependent drop in eosinophil, neutrophil, lymphocyte, and macrophage levels in BALF, indicating

Table 3. The effects of berberine on cell-mediated immunity.

Main effects	Experimental model	Dosage	Administration mode	Administration duration	Reference
Reduction of OVA-induced eosinophil, neutrophil, macrophage, and lymphocyte levels in BALF	Male Wistar rats	100 and 200 mg/kg	Oral	28 days	[29]
Suppression of CS-induced inflammatory-cell infiltration in alveolar lung tissue Reduction of CS-induced rise in macrophage, neutrophil and total cells in BALF	Male C57BL/6 mice	50 mg/kg	Intragastric	1 hr before CS exposure on 4 consecutive days	[32]
Attenuation of CS-induced airway histopathological changes Reduction of CS-induced goblet cell hyperplasia in lungs Reduction of CS-induced Muc5ac synthesis Reduction of CS-induced inflammatory cell influx to BALF	Male BALB/c mice	5 and 10 mg/kg	Intraperitoneal	30 min before CS exposure (twice daily, 6 days per week for 4 weeks)	[43]
Reduction of CSE-induced histopathological changes of lung tissue Reduction of CSE-induced rise in total and differential cell count in BALF	C57BL/6 mice	25 mg/kg (low dose) and 50 mg/kg (high dose)	Oral	Treatment for 6 days a week for 60 days	[30]
Attenuation of bleomycin-induced histopathological changes in lungs Suppression of bleomycin-induced inflammatory cell infiltration in BALF Suppression of bleomycin-induced MPO levels Suppression of bleomycin-induced mast cell deposition and histamine release	Male Wistar rats	200 mg/kg/day	Intraperitoneal	Treatment from days 1–14 (preventive group) and from days 14–28 (therapeutic)	[44]
Greater reduction of LPS-induced rise in lung W/D ratio with THBru than with berberine in mice	THP-1 cells	1, 5, and 10 μM	-	Pretreatment for 1 hr prior to LPS stimulation	[45]
Greater reduction of LPS-induced rise in protein content in BALF with THBru than with berberine in mice Greater reduction of LPS-induced rise total cell count and MPO level in BALF with THBru than with berberine in mice Reduction of LPS-induced lung structure damage with THBru and berberine in mice Reduction of LPS-induced rise in TNFα NO levels with THBru in THP-1 cells Downregulation of LPS-induced rise in JNK, AKT/p65, and p38 expression with THBru in THP-1 cells	Male ICR mice	50 mg/kg (Berberine) 2, 10, and 50 mg/kg (THBru)	Oral	Pretreatment for 1 hr prior to LPS stimulation	
Suppression of LPS-induced rise in macrophage, neutrophil, and total cell count in BALF in mice Reduction of LPS-induced rise in MPO activity	Male C57BL/6 mice	10 mg/kg	Intraperitoneal	Pretreatment twice (at 24 and 2 hrs before LPS stimulation)	[31]
Attenuation of pulmonary fibrosis Suppression of BLM-induced rise in phagocytic and inflammatory cells infiltration	Male Wistar rats	200 mg/kg/day	Intraperitoneal	Treatment for 14 days	[46]

reduced inflammatory infiltration in the airways. In accordance, in another *in vivo* investigation, berberine was found to ameliorate CS-induced lung injury via exerting potent anti-inflammatory effects [32]. CS is considered as one of the main risk factors involved in the pathogenesis of COPD and is known to trigger lung inflammation by initiating the infiltration of innate and adaptive inflammatory cells into the airways [50]. Berberine treatment (50 mg/kg) of C57BL/6 male mice was found to suppress the CS-induced rise in macrophage, neutrophil, and total cell count in BALF fluid, and was even found to significantly inhibit their infiltration into alveolar spaces, indicating reduced interstitial

edema and inflammation. The role of berberine in attenuating CS-induced lung inflammation was also confirmed in another *in vivo* study by Xu and colleagues [43]. Specifically, treatment of CS-induced male BALB/c mice with berberine (5 and 10 mg/kg) significantly attenuated the CS-induced airway histopathological changes, whereby it reversed the thickening of the airway epithelium, the obstruction of airway lumen by mucus and cell debris, and inhibited the infiltration of inflammatory cells, including neutrophils, which are regarded as the main inflammatory cells involved in the pathogenesis of COPD [51]. Additionally, berberine (5 and 10 mg/kg) dose-dependently inhibited the CS-induced increase in goblet cell hyperplasia and further molecular analysis revealed a significant drop in Muc5ac, the predominant mucin gene expressed in goblet cells, indicating reduced mucus production in mice airways. Importantly, a significant attenuation in CS-induced inflammatory cell influx and inflammatory cytokine release in BALF was also observed with berberine treatment (5 and 10 mg/kg). In particular, berberine treatment reduced CS-induced recruitment of total cells and differential cells to BALF and suppressed the influx of the proinflammatory cytokines and chemokines like TNFα, IL-1β, and MCP-1 into BALF. Suppression of such cytokines may have a substantial therapeutic effect on COPD as these cytokines have been shown to be associated with increased Muc5ac production, emphysema, and airway remodeling, all of which exacerbate COPD outcomes [52–54]. In another *in vivo* study, greater insight into the molecular mechanisms underlying the berberine-mediated attenuation of airway inflammation in COPD-induced mice was made [30]. In particular, treating C57BL/6 mice with berberine (low dose: 25 mg/kg; high dose: 50 mg/kg) prior to inducing them with cigarette smoke extract (CSE), a main risk factor for COPD, was found to significantly alleviate damage to bronchial lung tissue in CSE-induced mice by decreasing airway epithelium thickening, alveolus enlargement, and inflammatory cell infiltration. Additionally, high-dose berberine treatment (50 mg/kg) resulted in reduced total- and differential-cell count in BALF in CSE-induced mice. In another *in vivo* study, berberine was demonstrated to exert promising anti-inflammatory and anti-fibrotic effects against bleomycin-induced pulmonary fibrosis in a biphasic manner [44]. Specifically, treatment of bleomycin-induced male Wistar rats with berberine (200 mg/kg/day), at both a preventive (administered on the initial 1–14 days) and therapeutic (administered from days 14–28) mode reversed bleomycin-induced lung injury and suppressed histopathological damage to the lungs in rats. Additionally, berberine (200 mg/kg/day) treatment was also found to significantly inhibit mast cell production, histamine release, neutrophil, macrophage, and lymphocyte cell influx in BALF, and suppress MPO levels, confirming reduced neutrophil accumulation in BALF.

In another combined *in vivo* and *in vitro* investigation, Yu and colleagues explored the potential use of tetrahydroberberrubine (THBur), a berberine derivative with higher oral bioavailability, in alleviating ALI in male ICR mice and in the human monoblastic leukemia cell line, THP-1 [45]. As indicated previously, ALI and its more severe form, ARDS, are characterized by lung edema and neutrophil and inflammatory cell accumulation in the lung interstitium, possibly as a result of alveolar-capillary barrier disruption [55]. Treatment with an anti-inflammatory agent like berberine or its derivative, THBur, was found to improve ALI outcomes via decreasing various inflammatory mediators and signaling pathways that lead to the expression of inflammatory cytokines and chemokines. In particular, *in vivo*, treatment of LPS-induced mice with berberine (50 mg/kg) or THBur (2, 10, and 50 mg/kg) was found to curb down the LPS-induced damage caused to lungs, whereby a reduction in interstitial edema, thickening of the alveolar wall, and infiltration of inflammatory cells was observed. Additionally, analysis on inflammatory indices revealed a reduction in LPS-induced rise in wet to dry (W/D) ratio, protein and total cell count in BALF, and MPO content in mice lungs. Interestingly, these indices were found to be more potently reduced with THBur than with berberine, possibly due to its higher oral bioavailability. Similar promising results were also observed *in vitro* whereby THBur (1, 5, and 10 μM) significantly reduced LPS-induced rise in TNFα and NO expression, the latter of which is believed to contribute to enhanced pulmonary microvascular permeability and a greater likelihood for lung edema [56]. THBur was found to attenuate such inflammatory indices via downregulating the expression of MAPK p38 (only at 10 μM), AKT/p65, and c-Jun N-terminal kinase (JNK) signaling pathways. The protective effects berberine has on acute lung injury (ALI) were investigated in another *in vivo* study by Liang and colleagues [31]. Specifically, dexamethasone (DXM) and berberine treatment (10 mg/kg) of LPS-induced male C57BL/6 mice was found to be associated with a significantl decrease in lung edema, vascular permeability, total cell count in BALF, and MPO levels, indicating reduced neutrophil abundance in BALF. In another *in vivo* analysis, berberine was found to attenuate pulmonary fibrosis [46], a heterogeneous disease characterized by excessive deposition of extracellular matrix (ECM) within the pulmonary interstitium [57]. Specifically, treatment of bleomycin (BLM)-induced male Wistar rats significantly reduced lung damage, whereby a drop in fibroblast proliferation, alveolar damage, and pulmonary interstitial accumulation was observed in rats treated with berberine (200 mg/kg/day), as compared to those in the control group. Additionally, a significant dampening in phagocytic and inflammatory cell infiltration was also observed in the lung interstitium, indicating reduced pulmonary inflammation. Interestingly, the above parameters were found to be more potently reduced when a combination of berber-

ine (200 mg/kg/day) and DXM, an antifibrotic agent, was used, instead of either of those treatments alone, suggesting possible synergism between these two compounds. Future research should therefore be aimed at investigating compounds that may improve the efficacy of berberine in its fight against inflammatory pulmonary disorders.

5. Effects of Berberine on Other Inflammatory Mediators

The reported *in vivo* and *in vitro* effects of berberine on other inflammatory mediators involved in respiratory disorders are summarized in Table 4 (Ref. [29–31,44,58–60]).

Besides the modulatory effects of berberine on cytokine storm development, Th1/Th2 balance, and cell-mediated immunity, studies suggest that berberine may also help alleviate inflammation in various respiratory disorders via exerting inhibitory and modulatory effects on other aspects of innate and adaptive immunity. For example, in an *in vivo* investigation, berberine (100 and 200 mg/kg) was found to result in a significant and dose-dependent drop in immunoglobulin-E (IgE) levels in OVA-induced male Wistar, indicating reduced hyperinflammation [29]. In another *in vitro* study, berberine was found to suppress IgE-mediated hypersensitivity reactions in allergic disorders like asthma in rat basophilic leukemia cells (RBL-2H3) [58]. Specifically, pre-treating RBL-2H3, known to possess similar characteristics to mast cells, with berberine (0.3, 3, and 30 μM) prior to IgE sensitization using dinitrophenol-IgE human serum albumin (DNP-IgE/HAS) significantly curbed down β-hexosaminidase (β-HEX) and histamine release, indicating inhibition of mast cell degranulation. Additionally, berberine (0.3, 3, and 30 μM) treatment resulted in a significant attenuation in the levels of the key proinflammatory cytokines, TNFα and IL-4 in DNP-IgE/HSA-induced RBL-2H3 cells. Interestingly, molecular analysis revealed a significant downregulation in MAPK signaling, including (JNK), extracellular signal regulated kinase (ERK), and p38 pathways, and a reduction in FcεRI-mediated signaling in DNP-IgE/HSA-induced RBL-2H3 with berberine (0.3, 3, and 30 μM) treatment, indicating suppression of IgE binding to mast cell and hence a greater reduction in the cascade of the inflammatory pathways to follow.

Multiple studies also indicated that berberine may have the potential to attenuate cytokine storm development and progression via dampening the production and expression levels of not just cytokines, but also other inflammatory indices that seem to increase following lung infection. For example, in an *in vitro* study, berberine was found to exert protective anti-inflammatory effects against thymic stromal lymphopoietin (TSLP), an IL-7-like cytokine molecule believed to play a pivotal role in allergic disorders like asthma and COPD, in the human mast cell-line 1 (HMC-1) and in murine primary cultured bone marrow-derived mast cells (BMMCs) [59]. In particular, berberine (0.1–10 μM) pre-treatment was found to be associated with a significant drop in both TSLP production and mRNA expression levels in PMA+A23187-induced HMC-1 cells. A similar effect was also observed in BMMC, whereby berberine (10 μM) pre-treatment prevented the PMA+A23187-induced rise in TSLP production levels. Additionally, berberine (10 μM) was found to suppress caspase-1 activity, which has a role in cleaving IL-1β and IL-18 from their inactive forms to their active forms, indicating the possible role of berberine in reducing exaggerated cytokine expression in allergic disorders. Similar to other studies, berberine was found to exert its promising anti-inflammatory effects against allergic disorders via downregulating NF-κB expression, highlighting the role of this pivotal pathway in inflammation, including the development and progression of different types of inflammatory pulmonary disorders. In another combined *in vivo* and *in vitro* study, berberine was demonstrated to exert protective effects against ALI [31]. *In vitro*, treatment of LPS-induced human bronchial epithelial cells (16HBE) with berberine (5 and 10 μM) was found to be associated with a significant attenuation in endoplasmic reticular (ER) stress, a characteristic feature contributing to severe ALI symptoms. The berberine-mediated attenuation of ER stress in 16HBE cells occurred, partly, due to an increase in the nuclear translocation and expression levels of the nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor known for its anti-inflammatory effects by binding to antioxidant response element (ARE), an enhancer sequence known to orchestrate the expression of myriads of antioxidant genes [61]. The rise in the anti-inflammatory factor Nrf2 was also accompanied with a subsequent elevation in heme oxygenase-1 (HO-1) levels, which has a central role in the defense against oxidative and inflammatory insults in the lungs [62], in berberine treated (5 and 10 μM) and LPS-induced 16HBE cells. Additionally, similar to the findings observed *in vitro*, *in vivo*, DXM and berberine (10 mg/kg) treatment was found to attenuate inflammation in ALI in LPS-induced male C57BL/6 mice via elevating Nrf2 levels and dampening ER stress levels. Collectively, these findings point to the role of berberine in attenuating pulmonary diseases characterized by inflammation and dysregulated immune responses, like ALI and ARDS. In another *in vivo* study, berberine was demonstrated to exert promising anti-inflammatory and anti-fibrotic effects against bleomycin-induced pulmonary fibrosis in a biphasic manner [44]. Moreover, similar to results reported by Liang and colleagues (2019), berberine was found to attenuate oxidative stress parameters by upregulating Nrf2 expression. Importantly, berberine (200 mg/kg/day) also downregulated NF-κB inflammatory signaling pathway and its associated downstream cytokines and chemokines, including TNFα and inducible nitric oxide synthase (iNOS), and the anti-fibrotic agent transforming growth factor beta 1 (TGF-β1). In accordance, in another *in vivo* study, berberine was found to attenuate airway inflammation in CSE-

Table 4. The effects of berberine on other inflammatory mediators.

Main effects	Experimental model	Dosage	Administration mode	Administration duration	Reference
Reduction of OVA-induced IgE secretion	Male Wistar rats	100 and 200 mg/kg	Oral	28 days	[29]
Reduction of DNP-IgE/HSA-induced rise in B-HEX and histamine levels Reduction of DNP-IgE/HSA-induced rise in TNFα and IL-4 Reduction of DNP-IgE/HSA-induced FcεRI-mediated signaling Reduction of DNP-IgE/HSA-induced JNK, ERK, and p38 signaling	RBL-2H3 cells	0.3, 3, and 30 μM	-	Pretreatment for 1 hr prior to DNP/IgE-HSA stimulation	[58]
Reduction of PMA plus A23187-induced rise in TSLP production and mRNA expression levels Reduction of PMA plus A23187-induced rise in NF-κB expression in HMC-1 cells Reduction of PMA plus A23187-induced activation of caspase-1 activity Reduction of PMA plus A23187-induced rise in TSLP production in BMMC cells	HMC-1 and BMMC cell lines	0.1–10 μM	-	Pretreatment for 2 hrs	[59]
Promotion of Nrf2 nuclear translocation and phosphorylation in 16HBE cells	16HBE cells	2.5, 5, and 10 μM	-	Pretreatment for 4 hrs	[31]
LPS-induced drop in HO-1 levels Elevation of LPS-induced drop in Nrf2 levels in mice Reduction of ER stress levels in mice	Male C57BL/6 mice	10 mg/kg	Intraperitoneal	Pretreatment twice (at 24 and 2 hrs before LPS stimulation)	
Upregulation of Nrf2 transcription factor and an increase in antioxidant ability Suppression of NF-κB transcription Suppression of TNFα, iNOS, and TGF-β1.	Male Wistar rats	200 mg/kg/day	Intraperitoneal	Treatment from days 1–14 (preventive group) and from days 14–28 (therapeutic)	[44]
Downregulation of CSE-induced rise in TGF-β1/Smad mRNA and protein expression levels	C57BL/6 mice	25 mg/kg (low dose) and 50 mg/kg (high dose)	Oral	Treatment for 6 days a week for 60 days	[30]
Reduction of bleomycin-induced structural modification in lung tissue Reduction of bleomycin-induced smad 2/3 expression Reduction of bleomycin-induced TGF-β1 expression	Male Wistar albino rats	200 mg/kg body weight	Intraperitoneal	Treatment once daily for 27 days	[60]

induced mice via downregulation of TGF-β1/Smad signaling pathway, as a significant drop in TGF-β1, smad-2, and smad-3 mRNA and protein expression levels was observed in the berberine-treated group (50 mg/kg) [30]. The TFG-β1/smad pathway has been previously shown to be heavily involved in the pathogenesis of pulmonary fibrosis and asthma [63]. However, results from this study implicate the potential involvement of this pathway in the pathogenesis of COPD as well. The inhibitory potential of berberine on TGF/β1 and smad-2/3 expression levels was confirmed

in another *in vivo* study conducted on male Wistar albino rats [60]. The inhibitory effects of berberine on TGF-β1 were also confirmed in another *in vivo* study. In particular, administration of berberine (200 mg/kg body weight) to bleomycin-induced rats at both a preventive (administered on the initial 1–14 days) and therapeutic (administered from days 14–28) mode significantly reduced TGF-β1-mediated smad- and non smad- signaling cascades, indicating reduced expression of inflammatory mediators inflammatory cell influx, and restored bronchial integrity. In fact, ultrastructural analysis revealed restored normal alveolar structure and inhibition of abnormal interstitial tissue accumulation in berberine treated (200 mg/kg body weight) and bleomycin-induced rats, as compared to those only induced with bleomycin. Collectively, findings from all three studies implicate the involvement of TGF-β1/smad pathway in inflammation in many airway ailments, and the potential role berberine may have in suppressing such pathway. Although these studies looked specifically at the anti-inflammatory effects of berberine brought about by TGF-β1/smad downregulation, it is possible that downregulation of such pathway may be linked to other favorable effects in respiratory infections, such as preventing airway remodeling. Future studies should therefore discern the multifaceted role the TGF-β1/smad pathway may have on respiratory disease alleviation.

6. Potential Anti-Inflammatory and Immunomodulatory Role of Berberine in COVID-19

The above sections highlight the role immune dysfunctions play in different types of respiratory ailments and propose berberine as a promising candidate capable of alleviating such complications. Like many of these respiratory conditions, SARS-CoV-2 is also known to primarily target the airways and lung tissue [64]. Although the exact pathophysiological processes underlying COVID-19 are still under elucidation, multiple lines of evidence highly implicate immune dysregulations in COVID-19 pathogenesis and progression [65]. Indeed, studies revealed an increase in multiple inflammatory indices including C-reactive proteins (CRP), white blood cell count, neutrophil count, IL-6, and even ARDS in patients infected with COVID-19 [66,67]. The reported rise in inflammatory indices is believed to be mediated, at least in part, via COVID-19-induced dysregulations in immune responses [65]. Precisely, SARS-CoV-2 infection is thought to distort the balance between Th1/Th2 immune responses [68]. As such, the levels of Th1-related cytokines like TNFα and IL-2, and Th2-related cytokines like IL-4 and IL-10 were found to be elevated in the lung tissue following COVID-19, resulting in inflammation and cytokine storm development [69]. Given the involvement of immune dysregulations in many respiratory conditions and COVID-19, and the relevance of the organ system these conditions are mainly afflicting, it is tentatively suggested that the immunomodulatory effects exerted by berberine and outlined in the aforementioned sections may also extend to COVID-19. Specifically, it is suggested that berberine may help prevent and/or treat COVID-19 by preventing cytokine storm development, maintaining Th1/Th2 balance, preventing inflammatory cell-infiltration in lung tissue, and modulating other aspects of innate and adaptive immunity.

7. Direct Evidence for the Role of Berberine in Preventing and Treating COVID-19

It is clear that immune dysfunctions are highly implicated in COVID-19 and that such an infection could potentially be prevented, ameliorated, or even treated using anti-inflammatory and immunomodulatory agents. Despite the well-documted anti-inflammatory and immunomodulatory effects of berberine presented above, to date, literature exploring the immunomodulatory potential of berberine specifically against COVID-19 is rather scarce. Nevertheless, evidence from the studies that have been published so far on this topic are very promising and encourage future research in this area. Specifically, evidence from an *in vitro* study conducted by Wang and colleagues found that treatment of a SARS-CoV-2-infected Calu-3 cell-line with an immunotherapeutic berberine nanomedicine molecule named NIT-X (20 and 40 μg/mL) significantly curbed down hyperinflammation as the levels of IL-1α, IL-6, IL-8, and C-C motif chemokine ligand 2 (CCL-2) were reduced, indicating that berberine may be used to inhibit excessive pro-inflammatory cytokine expression and tissue damage during viral infection [70]. Analysis from another molecular docking and network pharmacology study found that berberine is capable of alleviating pneumonia pulmonary fibrosis in COVID-19 [71]. Pulmonary fibrosis is highly implicated as a serious complication of end-stage COVID-19 and is mainly characterized by fibroblast proliferation and extracellular matrix accumulation in the lung tissue [72]. Results from this study found that berberine was able to reverse COVID-19 pulmonary fibrosis via suppressing TNFα, inhibiting the synergistic effects between IL-6 and STAT3, and preventing CCL2 chemotaxis to fibrobloasts. In turn, these findings suggest the role berberine plays in suppressing inflammatory reactions and preventing fibroblast activation. Although preliminary, these results strongly support the anti-inflammatory propensity of berberine in COVID-19 alleviation. Future research should be directed at further substantiating these promising findings *in vitro* and *in vivo*. In another *in vivo* experimental investigation by Zhang and colleagues, berberine treatment was found to be associated with promising anti-inflammatory effects in 35 patients with severe COVID-19 symptoms and diarrhea [73]. In particular, berberine treatment (900 mg daily for 14 days) was found to significantly improve changes in IL-6, TNFα, and CRP levels in patients receiving both berberine and routine therapy, as compared to those receiving routine

therapy alone [73]. Intriguingly however, the reduction in cytokine and inflammatory marker levels was not observed in COVID-19 patients not suffering from diarrhea. Zhang and colleagues speculated that the improvement in COVID-19 patients with diarrhea could be partly explained by the protective effects berberine exerts on gastrointestinal function, which is believed to be not just a target for COVID-19, but also a source of inflammatory mediator production, thereby increasing the likelihood of cytokine storm initiation and progression. Future studies are certainly warranted to confirm this observation. Additionally, further research is required to further substantiate the immunomodulatory and anti-inflammatory potential of berberine in COVID-19.

In addition to the literature published specifically on the potentially promising immunomodulatory and anti-inflammatory effects of berberine in COVID-19 prophylaxis and treatment, substantial research has been conducted confirming the antiviral capacity of berberine against the dreaded disease COVID-19. Since the start of the pandemic, a multitude of antiviral drugs have been tested for their potential application as anti-COVID drugs. Of relevance, Remdesivir, Paxlovid, and Molnupiravir were all shown to demonstrate promising therapeutic effects against COVID-19 [74]. Of these 3 drugs, Remdesivir is the only drug currently approved by the FDA for the treatment of COVID-19 [74]. The latter two antiviral drugs are currently authorized by the FDA only for emergency use for COVID-19 treatment [74]. Despite the potentially promising therapeutic potential of such drugs in COVID-19 treatment, considerable adverse effects may accompany their use. For example, Paxlovid is known to exhibit significant and complex drug-drug interactions and has been shown to cause myalgia, dysgeusia, and diarrhea in COVID-19 patients [74]. Similarly, Molnupiravir was shown to be associated with diarrhea and nausea symptoms, in addition to a potential risk of genotoxicity in pregnant women [74]. As for Remdesivir, some studies have shown that its use can be linked to renal and liver toxicity, an elevation in the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), hypersensitivity, and nausea [74]. Given the plethora of side effects associated with the use of synthetic drugs, the use of a phytonutrient-derived antiviral agent, such as berberine, may prove to be especially useful. Berberine has previously been shown to be useful for the mitigation of a plethora of viral infections via exerting potent anti-inflammatory and immunomodulatory effects [28], and it is tentatively suggested that berberine may exert similar potent effects on SARS-CoV-2 virus. Literature published on the potential therapeutic effects of berberine against the dreaded disease COVID-19 is promising. For example, in an *in-silico* study conducted by Narkhede and colleagues, berberine was found to exert protective antiviral effects against COVID-19 [75]. In particular, berberine was found to be capable of exerting inhibitory effects on main protease (M^{pro}), a key protease in SARS-CoV-2 involved in promoting viral replication in the host. In fact, findings from molecular docking studies revealed that out of 12 compounds screened for potential M^{pro} binding abilities (glycyrrhizin, bicylogermecrene, tryptanthrine, β-sitosterol, indirubin, indican, indigo, hesperetin, crysophanic acid, rhein, berberine and β-caryophyllene), berberine was demonstrated to exert the third highest binding affinity (–8.1 Kcal/mol) to SARS-CoV-2 M^{pro}, indicating its promising potential in attenuating coronavirus replication. In accordance, besides its potential inhibitory effects on M^{pro}, berberine was also demonstrated to exhibit an inhibitory effect on two other potential targets of SARS-CoV-2, namely spike (S) protein and Angiotensin-converting enzyme-2 (ACE2) receptor. Like M^{pro}, S protein plays a critical role in modulating viral replication and transmission whereas ACE2 acts as the main receptor wherein S protein binds and infects host cells. As such, binding to one of these targets may prove to be useful for halting or slowing down SARS-CoV-2 infectivity in humans. Findings from a molecular docking analysis by Maurya and colleagues indicated that berberine demonstrated a significant binding affinity to both S protein and ACE2 receptor, suggesting that this potent natural isoquinoline may be beneficial to halt SARS-CoV-2 replication process [76]. These results were also corroborated in another molecular docking analysis conducted by Lakshmi and colleagues, whereby berberine was found to exert promising inhibitory effects on all SARS-CoV-2 targets [77]. Specifically, out of 47 bioactive compounds screened for their inhibitory effects on S protein, M^{pro}, and ACE2 rceptor, berberine was demonstrated to exert the 7th strongest binding energy to M^{pro} (–83.2 Kcal/mol), and the 2nd strongest binding energy to S protein and ACE2 receptor (–69.7 Kcal/mol and –71.5 Kcal/mol, respectively). Collectively, these results strongly suggest that berberine would certainly find its way into the arsenal of antiviral drugs targeting SARS-CoV-2. A summary of the proposed mechanisms of action of berberine against SARS-CoV-2 entry and replication is presented in Fig. 1. Although promising, future *in vitro* and *in vivo* studies are certainly warranted to ensure the inhibitory effects of berberine on M^{pro}, spike, and ACE2 targets in humans. Additionally, once confirmed in *in vivo* systems, it may be beneficial to look at the potential coupling of berberine with nanoparticles to further enhance its antiviral effects against SARS-Cov-2. Notably, a recent investigation suggests enhanced inhibitory effects on SARA-CoV-2 entry, replication, and assembly with the use of a berberine zinc-oxide nanoparticle complex than with berberine alone [78], suggesting promising synergistic effects between berberine and other compounds against COVID targets.

In another combined computational and *in vitro* study, Wang and colleagues indicated the potential therapeutic mechanisms of berberine in preventing and treating SARS-CoV-2 [70]. In particular, data from molecular docking analysis identified berberine as an efficient inhibitor

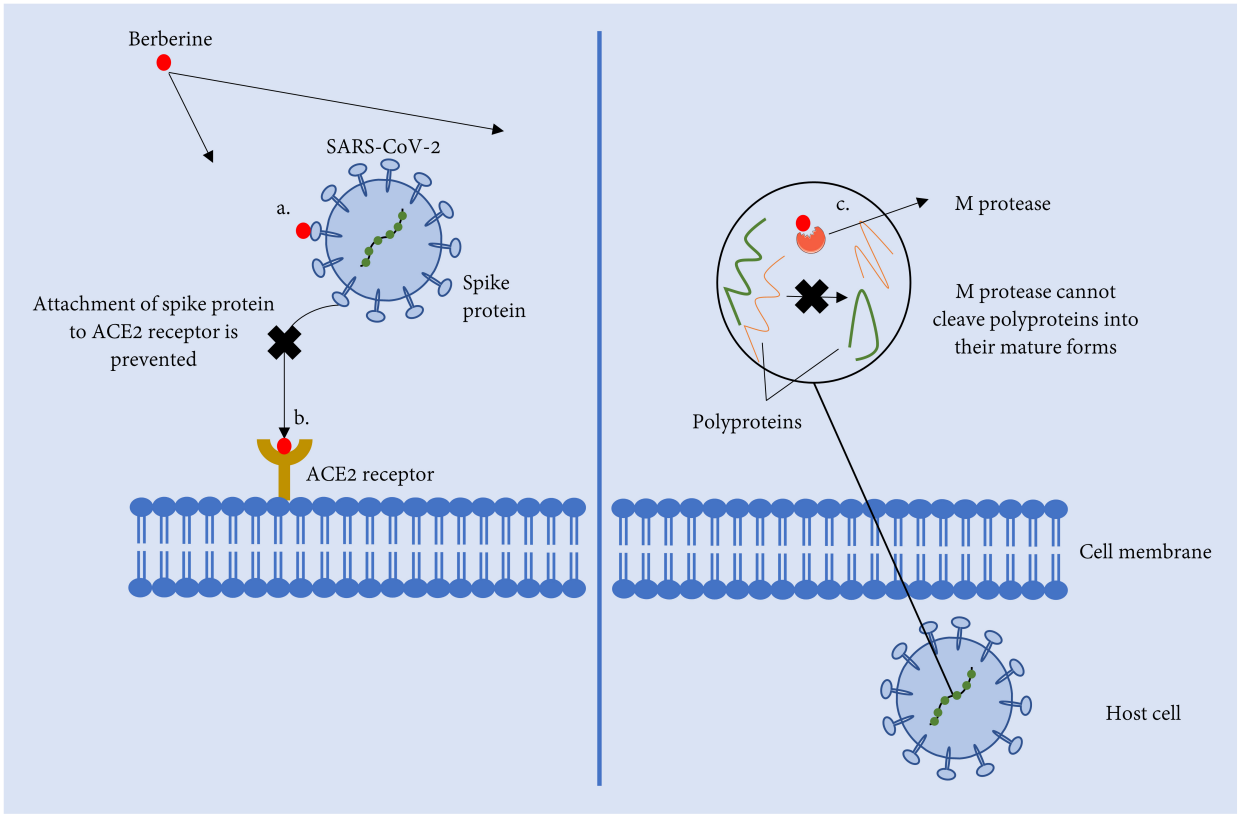

Fig. 1. Process of SARS-CoV-2 entry and replication in host cell and the therapeutic role of berberine against this process. Berberine may prevent SARS-CoV-2 entry to host cell via (a) attaching to spike protein, and/or (b) attaching to ACE2 receptor. Berberine may also halt SARS-CoV-2 replication via (c) attaching to M protease and preventing cleavage of polyproteins 1a and 1ab into their mature forms.

for multiple SARS-CoV-2 protein targets, including host immune-related proteins, host receptors, and virus proteins, which may aid in halting or suppressing hyperinflammation, viral entry, and viral replication, respectively. Specifically, berberine was found to exert moderate to strong binding affinities (–6.4—9.8 Kcal/mol), with the strongest binding results obtained for MAPK-3 (binding affinity: –8.9 Kcal/mol) and MAPK-8 (binding affinity: –8.6 Kcal/mol) for immune-related proteins, ACE2 (binding affinity: –9.8 Kcal/mol) for host receptor proteins, and 3CLpro (binding affinity: –6.7 Kcal/mol) for viral proteins. *In vitro* investigation from the same study further substantiated the role of berberine as an efficacious drug against SARS-CoV-2. In particular, treating SARS-CoV-2-infected Calu-3 cells with an immunotherapeutic berberine nanomedicine molecule named NIT-X (20 and 40 μg/mL) successfully inhibited SARS-CoV-2 replication and suppressed ACE2 and transmembrane serine protease 2 (TMPSS2) gene expression, the latter of which promotes SARS-CoV-2 infection and spread throughout the host via facilitating spike protein fusion with the host cellular-membrane. Further analysis from another *in vitro* investigation found that berberine (4.7–150 μM) suppressed SARS-CoV-2 viral replication process in African green monkey Vero E6 kidney cells via reducing infectious viral titer, indicating reduced production of infectious viral particles [79]. Interestingly however, berberine was not found to be associated with any significant attenuation in the process of viral RNA replication in SARS-CoV-2, indicating that berberine possesses its therapeutic effects against SARS-CoV-2 infection by acting mainly on the later stages of the SARS-CoV-2 life cycle. The same study also reported promising antiviral effects for berberine against SARS-CoV-2 in human nasal epithelial cells, which are believed to be more representative of the natural target cells (i.e., host cells). In particular, berberine was found to be effective at inhibiting SARS-CoV-2 RNA levels in the supernatant of the human nasal epithelial cell line with an EC_{50} value of 10.7 μM, suggesting its

potency at low concentrations. Collectively, findings from this study propose repurposing berberine as a potential therapeutic option against COVID-19. Findings from another *in vitro* study conducted by Pizzorno and colleagues found that berberine may demonstrate promising antiviral effects against SARS-CoV-2 [80]. In particular, berberine was found to significantly suppress viral production in African green monkey Vero E6 kidney cells infected with SARS-CoV-2, with a 50% inhibitory concentration (IC_{50}) of 10.6 μM. Interestingly however, combining a host-directed drug like berberine with a viral-directed drug like remdesivir was found to result in strong antagonistic effects, whereby remdesivir curbed down the dose-dependent attenuation in SARS-CoV-2 production observed with berberine, and vice versa. Noteworthy, these findings are preliminary, and further research is certainly warranted on other more representative physiological models like airway cell lines for more informed conclusions to be made. Nevertheless, the possibility of synergism between berberine and other viral-targeted drugs is an interesting avenue to be explored for the possible treatment of COVID-19.

8. Conclusions

Berberine as a natural agent possessing potent anti-inflammatory and immunomodulatory properties is very promising. This natural isoquinoline alkaloid has been shown to aid in the mitigation and/or treatment of various ailments including allergic and respiratory disorders. COVID-19 has emerged as a very serious threat to global health and economy. This respiratory pathogen is mainly characterized by excessive inflammation and immune dysregulations. The multiplicity of pathophysiological features induced by SARS-CoV-2 highlights the relevance of combination therapy for more effective protection against COVID-19. Thus, the use of berberine as an adjuvant therapy in addition to vaccines currently approved for use against the dreaded disease COVID-19 appears sensible. Berberine may benefit COVID-19 patients mainly by lowering inflammation and regulating immune responses. Precisely, berberine may help dampen cytokine storm, restore Th1/Th2 balance, and enhance cell-mediated immunity. Additionally, this critical natural compound may prove to be useful for modulating the levels of various other inflammatory mediators implicated in respiratory infections. Moreover, evidence from *in silico* and *in vitro* studies suggests berberine as a promising candidate for exerting inhibitory effects on three main SARS-CoV-2 targets, namely Mpro, ACE2 receptor, and S protein. As it stands, there is ample evidence supporting the role berberine may play in alleviating immune dysregulations and excessive inflammation in COVID-19. Yet, further well-planned *in vivo* and clinical studies are warranted for validation.

Author Contributions

AFM, SMY, IAAY, and GKN performed the literature analysis and wrote the original draft of the article. AFM and SMY generated the tables and the figure. AFM, SMY, IAAY, and GKN critically reviewed and revised the final draft of the article.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest. GKN is serving as one of the Editorial Board of this journal. We declare that GKN had no involvement in the peer review of this article and has no access to information regarding its peer review.Full responsibility for the editorial process for this article was delegated to AA.

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