Review

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A new application of mTOR inhibitor drugs as potential therapeutic agents for COVID-19

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Abstract: Since December 2019, the COVID-19 emerging pandemic caused by SARS-CoV-2 has resulted in one of the most important global health threats. Concerning the absence of an approved effective vaccine or drug for the treatment and outcome improvement of COVID-19 patients, and the role of SARS-CoV-2 in activation of mammalian target of rapamycin (mTOR) pathway, we decided to review the previous data regarding the therapeutic effect of mTOR inhibitor drugs in COVID-19 patients. We searched the scientific databases such as Web of Science, Embase, Medline (PubMed), Scopus, and Google Scholar using appropriate keywords to find suitable studies or suggestions until October 2020. The findings of the current study confirmed that mTOR inhibitor drugs through suggested mechanisms such as T cell adjustment, induction of autophagy without apoptosis, reduction of viral replication, restoration of the T-cell function, decrease cytokine storm, and moderation of the mTOR–PI3K–AKT pathway activation bring about a therapeutic impact in COVID-19 patients. Taken together, it is necessary to find a suitable therapy for the COVID-19 pandemic emerging. In this regard, we clarify that it is valuable to consider the therapeutic effect of mTOR inhibitor drugs and metformin by its mTOR inhibition property in the treatment of COVID-19 patients.

Keywords: coronavirus; COVID-19; metformin; mTOR inhibitors; rapamycin; SARS-CoV-2; sirolimus.

Introduction

Coronaviruses (COVs) are a large subspecies of the Coronaviridae family causing a wide range of diseases from the common cold to more severe and deadly diseases such as severe acute respiratory syndrome coronavirus (SARS-CoV, between the years 2002 and 2003), the middle east respiratory syndrome coronavirus (MERS-CoV, 2012 to present), and 2019-novel coronavirus (2019-nCoV, or COVID-19, from 2019 until now) infections in humans [1, 2]. For the first time, COVs as large, nonsegmented, enveloped, and single-stranded RNA viruses were discovered in the 1960s [3] and classified into alpha, beta, delta, and gamma genera between them, alpha and beta COVs possess the ability to infect humans [4].

At the end of 2019, the World Health Organization (WHO) announced a new sort of betacoronavirus named COVID-19 which emerged in Wuhan, China, and rapidly spreading around the world [5, 6]. COVID-19 (the infection caused by SARS-CoV-2) outbreak results in a greater threat of global health and varying degrees of clinical symptoms such as fever, dry cough, shortness of breath, hypoxemic respiratory failure, unusual radiologic findings, lymphopenia, myalgia, malaise, anosmia, acute kidney injury (AKI), and gastrointestinal manifestations [6–9].

The chief diagnostic test for COVID-19 is the detection of SARS-CoV-2 from a human nasopharyngeal swab or other clinical laboratory specimen using the real-time reverse transcription-polymerase chain reaction (RT-PCR) assay [7, 10]. However, chest computed tomography (CT) scan is another valuable test to distinguish the infection mentioned above [11, 12]. Moreover, patients admitted to ICU present higher levels of interleukin (IL)-2, IL-6, IL-7, IL-10, IL-1 β , tumor necrosis factor-alpha (TNF- α), granulocyte-colony stimulating factor (GSCF), monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory proteins (MIP), and IFN- γ -inducible protein 10 (IP10) in plasma [12–14].

The mammalian target of rapamycin (mTOR), known as a serine/threonine kinase, regulates cell growth, protein synthesis, cell proliferation, and cellular homeostasis. The surprising point is that the mTOR takes part in

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viral replication [15]. Recent studies have stated that the mTOR–PI3K–AKT signaling pathway is an important pathway in SARS-CoV-2 infection. RNA viruses such as SARS-CoV-2 stimulates this signaling pathway through activation of phosphatidylinositol 3-kinase (PI3K) and its downstream molecule AKT, or even by activating mTOR (Figure 1) [16–18]. Therefore, SARS-CoV-2 results in the generation of IL-1 β as a mediator of lung inflammation, fibrosis, and fever [19]. mTOR inhibitor drugs such as metformin, sirolimus, and everolimus can moderate the infection by blocking the viral genome transcription and protein synthesis [17]. So, mTOR inhibitors block the cell-cycle progression and suppress the proliferation of T cells due to the cross-link of antigenic peptides and/or cytokines like IL-2 with the T-cell receptors [20–22]. Furthermore, sirolimus could reverse the induction of IL-1 β production caused by binding of SARS-CoV-2 to toll-like receptor (TLR) [23].

Patients affected by COVID-19 must be quarantined and stayed at rest [24]. The main weakness in COVID-19 infection treatment is the absence of a specific vaccine or appropriate medicine. In consideration of that, WHO planned supportive cares for instance the co administration of oxygen therapy [25], mechanical ventilation [25], COVID-19 Convalescent Plasma [26, 27], and a wide range of therapeutic drugs such as antiviral agents (oseltamivir [11], remdesivir [28, 29], hydroxychloroquine [30]), and supporting agents (methylprednisolone [31],

tocilizumab [32], Anakinra [14], Ascorbic acid [25], and azithromycin [33]), along with the ACE inhibitors and angiotensin-receptor blockers (ARBs) [34]. Furthermore, it is suggested that the natural medicinal plants including Curcumin, *Camellia sinensis*, *Nigella sativa*, *Allium sativum*, *Hypericum perforatum*, and *Scutellaria baicalensis* through amelioration the immune response can play an important preventive and therapeutic role against COVID-19 infection [35, 36].

Nowadays, COVID-19 is the most significant health threat worldwide and causes severe systemic inflammation, acute respiratory distress syndrome (ARDS), and a high mortality rate. Concerning the role of SARS-CoV-2 in activation of the mTOR pathway and considering that no therapeutics have been proven beneficial for the treatment of a severe ailment induced by SARS-CoV-2, we aimed to review the prior researches to detect the therapeutic efficacy of mTOR inhibitor drugs in COVID-19 patients. It is noteworthy that a previous Focus article has written by Naserifar et al. [37] has briefly suggested the use of sirolimus and everolimus in clinical trials based on possible mechanisms, but in the present narrative review, we have studied and listed all previous clinical and preclinical studies accompanied by a letter to the editor, editorial, opinion, research perspective, and commentary articles regarding the use of mTOR inhibitor drugs for COVID-19 treatment. We have also reviewed metformin based on its mTOR inhibitor properties.

Figure 1: The effects of mTOR inhibitors on the mTOR–PI3K–AKT signaling pathway during SARS-CoV-2 infection. This figure was created by BioRender.com. mTOR: mammalian target of rapamycin; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, ACE2: Angiotensin-converting enzyme 2, PI3K: Phosphoinositide 3-kinases.

Method

The current literature search was conducted using scientific databases such as Web of Science, Embase (Excerpta Medica Database), Medline (PubMed), Scopus, and Google Scholar. In this academic search, the combinations of the following terms were used: “Coronaviridae family” and “SARS-CoV-2” and “COVID-19” and “coronavirus” and “betacoronavirus” and “coronavirus 2” and “coronavirus disease 2019” and “2019-nCoV disease” and “mTOR signaling” and “mTOR inhibitors” and “sirolimus” and “everolimus” “rapamycin” and “inflammation” and “cytokines”. In terms of increasing the search strategy accuracy, the wild-card asterisk symbol (*) was used. The bibliography of every article was assessed to identify and include suitable published studies (clinical or preclinical researches, letter to the editor, editorial, opinion, research perspective, and commentary) or ongoing Trials until October 2020 to notice the studies regarding protective effects of mTOR inhibitors on the outcome of the COVID-19 patients. Our narrative review included studies written in English.

Result and discussion

mTOR inhibitors

First, we will review the effect of mTOR inhibitor drugs such as sirolimus (rapamycin) and everolimus (RAD001) on the treatment of COVID-19 infection in patients and *in vitro*.

A careful hypothesis proposed about protective effect of combination therapy of lithium chloride plus rapamycin in patients who suffered from lung damage due to COVID-19. They stated these patients had possessed a deregulated response in the activity of T cells. It is suggested that COVID-19 may lead to unwanted replication of the virus through a delay in the interferon response, thereby sensitizing T cells to progress the macrophage stimulation and apoptosis. As a result of the high threshold of interferon-response in elderly patients, they are in a risk of COVID-19 pneumonia. However, there was a reliable suggestion for co-administration of rapamycin owing to mTOR inhibition and T cell adjustment, inducing autophagy without apoptosis along with lithium chloride due to inhibition of protein synthesis following inactivation of glycogen synthase kinase β . This process brings about a decrease in virus influx to cells and a diminution in viral RNA synthesis thereby diminishing the viral replication

ability. Correspondingly, it is introduced as an appropriate proposed treatment for COVID-19 infected patients. Notably, this is an essential issue for performing further clinical studies [38].

Concerning severe COVID-19 infection resulted in a decrease in counts and activity of T cells as well as cytokine storm syndromes in patients [39], Omarjee et al. [40] in a letter discussed the capability of sirolimus, as an mTOR inhibitor on restoring the T-cell function and reduction of a cytokine storm. They declared that rapamycin prescription at the beginning of the cytokine storm stage through the down-regulation of the senescent T-cell count, mTOR-NLRP3-IL-1 β axis, and IL-6 pathway lead to prevention of development the severe forms of COVID-19. Furthermore, the administration of rapamycin and antiviral drugs could develop an excellent treatment for COVID-19 patients who are involved in an advanced chronological stage with decreased T-cell counts.

A recent study regarding the evaluation of integrative proteo-transcriptomics to access the map of cellular response to the virus over time was performed on the HuH7 cells infected by SARS-CoV-2. The findings exhibited an alteration in the mTOR/HIF-1 signaling cascades at the proteo-transcriptomic analysis with the exact unclear mechanism in these infected cells. Besides, inhibition of mTOR signaling could be considered as a potent factor in the reduction of cytokine storm syndromes in critically infected patients with COVID-19. Notably, drugs targeting mTOR/HIF-1 signaling could be accounted as strong candidates, alone or in combination with antiviral drugs, for the treatment of COVID-19 patients [41].

Overall, there seems to be some evidence to indicate that COVID-19 enters the lung cells through both ACE2 and CD26 receptors which are related to senescence. The senescent cells play an important role in the production of inflammatory cytokines like IL-6. It can thus be suggested that anti-aging drugs such as rapamycin could inhibit protein synthesis and viral replication. Importantly, it could conceivably be hypothesized that rapamycin possesses an outstanding part in deterrence the virus transmission. Consequently, it could be considered as an appropriate treatment against COVID-19 infection. This research presented a review of the anti-aging therapeutics against COVID-19 disease and suggested a direction for future clinical trials in this regard [42]. One of the main drawbacks of this article was that rapamycin is an important inhibitor of mTOR signaling, but this study did not mention it.

The purpose of Zhavoronkov et al. [43] research paper was to review the recently available researches into the choice of a senoremediative and geroprotective interventions for better protection of elderly patients

against gerolavic infections. They expressed the assumed geroprotectors, specially sirolimus and everolimus (RAD001) owing to mTOR inhibition, diminished the infection grades in elderly patients.

The valuable *in silico* study was undertaken to find an effective antiviral drug against SARS-CoV-2 using a network-based drug repurposing model. The findings exhibited that sirolimus and dactinomycin can be considered as a potent combination treatment for the SARS-CoV-2 infected patients due to the inhibition of mTOR signaling and RNA synthesis pathway by DNA topoisomerase 2-beta (TOP2 β) and DNA topoisomerase 2-alpha (TOP2 α) [23].

Recently, the researchers, by using the L1000FDW web-based utility, recognized the potent drugs based on their biological pathways for the COVID-19 treatment and control disease progression in the primary human lung epithelium. Consequently, they proposed sirolimus as an mTOR inhibitor is a candidate drug for the treatment of COVID-19 patients [44].

Recently, Terrazzano et al. [45] in their short opinion suggested to clinicians the use of mTOR inhibitor drugs as great immune-regulating agents in suitable doses for the SARS-CoV-2 infection treatment. They assumed everolimus through inhibition of T lymphocytes and maintain the Treg activity can decrease hyper-reactivity in COVID-19.

mTOR protein participates in the regulation of cellular metabolism, cell proliferation, and protein synthesis. There has been evidenced that coronavirus infection through an increase in mTOR expression levels facilitated virus replication. Consequently, the mTOR inhibitors such as everolimus and rapamycin are capable to prevent the SARS-CoV-2 replication in human lung cells [46].

A Focus article based on important arguments such as immunomodulatory effects of everolimus, mTORC1 inhibitor impact of sirolimus, and thereby inhibition of the protein expression and virus release suggested that sirolimus prescription to COVID-19 patients in large-scale clinical trials can be considered as a standard treatment protocol in this regard [37].

Rapamycin, as an mTOR inhibitor drug at low dosages through inhibition of the cell proliferation, viral protein synthesis and replication, and expression or activities of pro-inflammatory cytokines including IL-2, IL-6, and IL-10 can be considered for COVID-19 treatment. Also, rapamycin owing to its anti-aging effects moderates the cytokine storm, and viral particle synthesis. It is notable that rapamycin due to targeting the host agents, not only viral machinery, can become a better candidate in comparison to antiviral drugs for COVID-19 treatment [47].

For the first time, Peron et al. [48] in 2020 assessed the presence and outcome of COVID-19 infection in 102

patients affected by lymphangioliomyomatosis (LAM) and/or tuberous sclerosis complex (TSC) and evaluated the efficacy of mTOR inhibitor drugs, including everolimus or sirolimus received by 26 of these patients. The findings showed that none of these patients on mTOR inhibitors was recognized with SARS-CoV-2 infection, and those with suspicion of respiratory symptoms of COVID-19 infection returned to health. To sum up, their study proposed the feasible role of mTOR inhibitors for COVID-19 infection treatment in patients with TSC.

What is now performing is a registered randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov number, NCT04341675.) involving 30 hospitalized patients who suffered from COVID-19 Pneumonia divided into two groups, receiving sirolimus or placebo. Sirolimus will be administrated 6 mg orally at first and followed by 2 mg daily for a maximum of 14 days duration time or up to hospital discharge. Regarding the aim of this study is the evaluation of improving the clinical outcomes of hospitalized patients infected with COVID-19, the clinical state, coadministration of drugs, and laboratory markers will be checked daily. The study-specific parameters will be measured at days 0, 3, 7, and 14 [49].

Walter K Kraft founded a randomized, double-blinded, placebo-controlled trial study (ClinicalTrials.gov number, NCT04371640) at Thomas Jefferson University, Philadelphia, the U.S, to evaluate the virological efficacy, safety, tolerability, pharmacokinetics, and pharmacodynamics of sirolimus adjuvant therapy in patients affected by COVID-19 from May 2020 to July 2020 on 40 participants (18 years up to 65 years old). He prescribed Rapamune (sirolimus) (Day 1: 10 mg, Days 2–7: 5 mg orally) in the treatment group and Placebo (Day 1: 10 mL, Days 2–7: 5 mL orally) in the control group. The primary outcome was specified as the alteration in SARS-CoV-2 viral load from baseline to day 7 of treatment duration measured by quantitative real-time polymerase chain reaction (qRT-PCR). The secondary outcome was defined as the alteration in SARS-CoV-2 viral load using qRT-PCR and the safety and tolerability of sirolimus in COVID-19 patients at days 1–6 of treatment. The final results of Walter's study are not published [50].

In another pilot, multicenter randomized open-label trial study (ClinicalTrials.gov number, NCT04374903) founded at King Hussein Cancer Center, Jordan, hydroxychloroquine 600 mg orally for 10 days in combination with oral sirolimus 4 mg for 1 day and then 2 mg daily for 9 days was prescribed to 58 COVID-19 patients (18 years and older) from May 1, 2020, to September 1, 2020. In the primary outcome assessment, they evaluated the time to clinical improvement in a time frame of 28 days. In the secondary

outcome measurement, the factors such as death or necessity of intubation and mechanical ventilation, safety and tolerability, QT interval prolongation, failure to continue assigned therapy, and time to viral clearance using RT-PCR in 28 days' time frame were evaluated [51].

Metformin

Metformin is a biguanide that activates the 5'AMP-activated protein kinase (AMPK) signaling pathway through the liver kinase B1 (LKB1). Therefore, the drug inhibits the mTOR pathway and decreases protein synthesis and cell proliferation without lung toxicity [52].

Hospitalized patients infected by COVID-19 with diabetes mellitus can provide challenges to clinicians in terms of treatment decisions. Here, we reviewed the studies that focused on the impact of metformin as an mTOR inhibitor on the outcome of hospitalized COVID-19 patients with diabetes.

Between January 27, 2020, and March 24, 2020, a retrospective observational study using a total of 283 COVID-19 patients (104 patients receiving metformin and 179 patients no-metformin intake) were performed at the Tongji Hospital of Wuhan, China, to assess the outcome in metformin users and non-users. The demographic characteristics, laboratory profiles, medications, and clinical findings were evaluated retrospectively. The finding provided evidence that metformin treatment contributed to a decrease in the hospital mortality rate in comparison to diabetic COVID-19 patients not receiving metformin. Among the plausible explanations for these findings is that

metformin may provide benefits in diabetic COVID-19 patients [53].

Most previous studies have focused on the antidiabetic properties of metformin; however, it is valuable to provide an insight into the antiviral ability of the mentioned drug. It is beyond the scope of this study that nowadays, metformin is introduced as the aspirin of the 21st century. The phosphorylation of AMPK in hepatocytes by metformin is the main mechanism of metformin on glucose and lipid metabolism. Consequently, metformin directly through LKB1 activates the AMPK and inhibits the mTOR pathway. Moreover, this drug indirectly via insulin receptor substrate 1 (IRS-1) phosphorylation modulates AKT pathway activation that brings about the mTOR signaling inhibition. As far as metformin is capable of inhibiting the pathway mentioned above, it could be concluded that this medicine will play an important role against SARS-CoV-2 [54].

Collectively, the prior studies outlined a critical role for metformin in aging-related mechanisms such as inhibition of mTOR and mitochondrial complex 1, as well as reduction of insulin-like growth factor-1 (IGF-1) levels. Recently, metformin was used in the targeting aging with metformin (TAME) study as the first extensive human clinical trial of aging for evaluation of its impact on the occurrence of a complex outcome in cancer, cardiovascular events, and dementia along with mortality. Their research presented an assessment and suggested a direction for more concentration on the effects of metformin on gerolavic and gerophilic infections in the background of the TAME study and further clinical trials [43]. The evidences assessing the therapeutic effects of mTOR inhibitor drugs on COVID-19 are summarized in Table 1.

Table 1: A summary of evidences investigating the therapeutic effects of mTOR inhibitors on COVID-19.

Type of evidence	Medication	Study design	Result/mechanism	Reference
Editorial	Lithium chloride plus rapamycin	–	A decrement in the possibility of viral replication. Regulating the immune response. Stimulating autophagy without apoptosis.	[38]
Editorial	Rapamycin	–	mTOR-NLRP3-IL-1 β axis down-regulation in the IL-6 ^a pathway. Down-regulation of senescent T-cell number.	[40]
Cellular experiment	Recommended mTOR inhibitors	HuH7 cells were infected with CoV-2 ^b at MOI ^b 1.0	Alteration in the mTOR/HIF-1 signaling cascades at the proteo-transcriptomic analysis by CoV-2. Reduction of cytokine storm syndromes by recommended mTOR inhibitors.	[41]

Table 1: (continued)

Type of evidence	Medication	Study design	Result/mechanism	Reference
Research perspective	Rapamycin	–	Inhibition the protein synthesis and viral replication.	[42]
Research perspective	Sirolimus and everolimus (RAD001)	–	Diminishing the infection grades in elderly patients.	[43]
Network-based methods study	Sirolimus plus dactinomycin	Using a network-based drug repurposing model and HCoV ^d –host interactions	Inhibition of mTOR signaling and RNA synthesis pathway by TOP2β ^e and TOP2α ^f .	[23]
Network-based methods study	Sirolimus	L1000FDW web-based utility in the primary human lung epithelium	Encouraging the therapeutic efficacy of mTOR inhibitors in fighting against COVID-19. Improving the symptomatology. Decreasing mortality rates.	[44]
Opinion	mTOR inhibitor drugs	–	Inhibition of T lymphocytes. Maintain the Treg activity.	[45]
Opinion	mTOR inhibitor drugs	–	Prevention of CoV-2 replication in human lung cells.	[46]
Focus	Sirolimus, everolimus	–	Immunomodulatory effects of everolimus. mTORC1 inhibitor impact of sirolimus.	[37]
Review	Rapamycin	–	Inhibition of the cell proliferation, viral protein synthesis and replication, and expression or activities of pro inflammatory cytokines including IL-2, IL-6, and IL-10. Moderation of the cytokine storm and viral particle synthesis.	[47]
Cohort study	Sirolimus, everolimus	– 102 patients affected by LAM ^g and/or TSC ^h – 26 patients received everolimus (2.5–10 mg/day) or sirolimus (1–5 mg/day)	Fully recovering in those with suspicion of respiratory symptoms of COVID-19 infection.	[48]
A randomized, double-blind, placebo-controlled trial	Sirolimus	– 30 hospitalized patients with COVID-19 – 15 patients receiving 6 mg sirolimus orally at first and then 2 mg daily for 14 days – 15 patients receiving placebo for 14 days	In processing	[49]
A randomized, double-blinded, placebo-controlled trial	Rapamune (sirolimus)	– 40 hospitalized patients with COVID-19 – Treatment group receiving oral sirolimus day 1: 10 mg, days 2–7: 5 mg, and the control group receiving oral placebo day 1: 10 mL and days 2–7: 5 mL	In processing	[50]
Pilot, multicenter randomized open-label trial	Hydroxychloroquine + sirolimus	– 58 hospitalized patients with COVID-19 – Hydroxychloroquine 600 mg orally for 10 days in combination with oral sirolimus 4 mg for 1 day and then 2 mg daily for 9 days or azithromycin 250 mg daily for 10 days	In processing	[51]
	Metformin	– 283 diabetic COVID-19 patients	A decrement in hospital mortality rates.	[53]

Table 1: (continued)

Type of evidence	Medication	Study design	Result/mechanism	Reference
Retrospective observational study		– 104 patients received metformin for at least 3 days and 179 patients not received metformin	Increment of benefits in diabetic COVID-19 patients.	
Commentary	Metformin	–	Phosphorylation and activation of AMPK ¹ . Phosphorylation of IRS-1 ¹ . Modulation of the AKT pathway. Inhibition of the mTOR pathway.	[54]
Research perspective	Metformin	TAME ^k	The suggestion of the effects of metformin on geriatric and gerophilic infections as mTOR inhibitor drugs.	[43]

^aInterleukin 6. ^bSARS-CoV-2. ^cMultiplicity of infection. ^dHuman coronavirus. ^etopoisomerase 2-beta. ^ftopoisomerase 2-alpha.

^glymphangiomyomatosis. ^htuberosclerosis complex. ⁱ5'AMP-activated protein kinase. ^jInsulin receptor substrate 1. ^kTargeting Aging with Metformin.

Conclusion

SARS-CoV-2, an emerging virus, has spread quickly in the world since December 2019, and WHO introduced COVID-19 as a pandemic infection. Until now, an approved effective vaccine or medicine has not been confirmed. In this regard, different available drugs are being introduced for the treatment of COVID-19 patients, along with several ongoing clinical trials that are carried out to evaluate their potential efficacy. To sum up, it is a fundamental necessity to find an executable therapy for the emerging COVID-19 outbreak. In this attempt, we suggest that it is valuable to reconsider the therapeutic properties of mTOR inhibitor drugs and metformin considering its mTOR inhibitor properties for the treatment of COVID-19 patients.

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