Role of Rho-kinase Inhibitor and hrsACE2 in COVID-19 Patients Management

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Abstract

The Renin-angiotensin system (RAS) plays a vital role in the SARS-CoV-2 infection through the Angiotensin-converting enzyme 2 (ACE2). Human recombinant soluble ACE2 (hrsACE2) has been shown to act as a competitive interceptor for SARS-CoV-2 by preventing the binding of viral particles to the ACE2. In addition, Rho-kinase inhibitors have been proven to suppress SARS-CoV-2 infection by inhibiting the Rho-associated protein kinase (ROCK) pathway. The combined effect of hrsACE2 and Rho-kinase inhibitor increases the activity and levels of ACE2 in protection through the Ang1-7 MasR, Ang1-9, and other mechanisms of protection. This literature review is done by searching journals with “COVID-19”, “ACE2”, “hrsACE2”, “Rho-kinase inhibitor”, and “ROCK pathway” as well as Boolean logic “AND” and “OR”. Relevant journals are used as references to compile systematic writing. Based on research results, hrsACE2 and Rho-kinase inhibitors have been clinically proven to protect tissue through the elevated level of ACE2 by the mechanism of Rho-kinase inhibitor, which increased Ang1-7 MasR concentration that gives vasodilatation, anti-proliferative, anti-inflammatory, and anti-fibrotic effect. Rho-kinase inhibitors significantly reduce the number of infected cells by SARS-CoV-2 in COVID-19 patients. The potential of the combination therapy of Rho-kinase inhibitor and hrsACE2 therapy can be an efficient therapeutic solution for COVID-19 patients so that further research can be carried out in the future.

Keywords: ACE2; COVID-19; hrsACE2; Rho-kinase inhibitor; Renin-angiotensin system; SARS-CoV2.

Introduction

COVID-19 is a disease caused by infection with the SARS-CoV-2 virus, which first appeared in Wuhan, China, in December 2019 and has become a global health problem. The high process of human-to-human transmission leads this virus to spread rapidly throughout the world. Based on WHO, the number of COVID-19 cases has reached 115,094,614 and 2,560,995 deaths in 223 countries, with Indonesia in the 18th place for the highest COVID-19 cases in the world with 1,368,093 cases and 37,000 deaths. Results showed angiotensin-converting enzyme 2 (ACE2) overexpression in most COVID-19 patients. ACE inhibitor (ACEi) class drugs as therapy have been considered. However, some concerns using ACEi class drugs and angiotensin II receptor

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blocks (ARBs) can increase the COVID-19 patients mortality.4 Previous antiviral drugs, such as remdesivir and favipiravir, can provide sound therapeutic effects for COVID-19 patients, but there are still some side effects.5,6 Currently, ACE2 has become the target of COVID-19 research and various drug development efforts.7 Human recombinant soluble ACE2 (hrsACE2), a genetically modified variant of ACE2, which plays a role in forming extracellular vesicles, plays a crucial role in transmitting SARS-CoV-2 via extracellular vesicles (EVs).8,9 Rho-kinase inhibitors regulate ACE2 by increasing ACE2 activity and levels in an experimental model of hypertension in pulmonary artery endothelial cells.

In looking for novel COVID-19 therapies, the potential for rhino-kinase inhibitors and hrsACE2 therapy combination to play a role in COVID-19 patients’ treatment is feasible; thus, the authors aim to provide a new theoretical basis for the treatment of COVID-19 patients.

Pathogenesis and Pathophysiology of COVID-19

Highly pathogenic CoV infections, including SARS-CoV-2, cause severe flu-like symptoms and develop into acute respiratory distress (ARDS), pneumonia, kidney failure, and death.10,11 SARS-CoV-2 incubation period in the body for a long time, namely 2-14 days.12,13 The transmission of SARS-CoV-2 from cell to cell-mediated by EVs through cell communication in both physiological and pathological conditions.14-16 EVs can transfer viral components, including genomic molecules, proteins, and receptors that come from infected cells, to infect other healthy cells.17

SARS-CoV-2 attaches to the ACE2, which is highly expressed in pulmonary epithelial cells and other tissues.18,19 Specifically, the S protein spike is responsible for binding to the ACE2.20 Protein S is used by the transmembrane serine two protease (TMPRSS2) on host cells cleaving ACE2 to support the fusion of SARS-CoV-2 with epithelial cells.21

Role of ACE2 in hydrolyzing AngII to angiotensin 1-7 (Ang1-7) and angiotensin I (AngI) to angiotensin 1-9 (Ang1-9)22,23 is crucial in preventing acute lung injury (ALI).24 In an ARDS patient, AngII also impairs adaptive immunity by activating macrophages and other immune system cells with increased production of IL-6, TNFα, and other cytokines. Most of the detrimental effects of AngII result from stimulation of AngII type 1 receptor (AT1R), which will be discussed further in pharmacodynamics.25

ROCK pathway has an essential role in stimulating vasoconstriction and vascular remodeling, activating G membrane proteins, one of which is AngII, by activating phospholipase-C, protein-kinase C, and myosin II regulatory chain phosphorylation, causing hypertension.26 By inhibiting the ROCK pathway, gene expression and activation of enzymatic ACE and AngII levels in plasma are reduced.27

HrsACE2 Therapy and Rho-kinase Inhibitor in COVID-19 Patients

Recent studies show hrsACE2 can block the initial entry of SARS-CoV-2 infection in alveolar type II epithelial cells by two mechanisms involving the binding of protein to neutralize SARS-CoV-2 and minimize injury to multiple organs.28 In a case report, it was found that the first severe COVID-19 patient was successfully treated through hrsACE2 therapy.29

Rho-kinase inhibitors have been tested to show moderate to solid inhibitory strength against ROCK1 and ROCK2.30 This inhibition will interfere with the Rho GTPase-dependent signaling pathway and disturb micro vesicle shedding. Rho-kinase inhibitors also play a role in AngII levels in ALI patients.31 Fasudil tested in vivo and in vitro on ALI provides sound clinical effects, including reducing pulmonary hypertension, inducing arterial vasodilation, decreasing pro-inflammatory cytokines, and reduced neutrophil infiltration in lung tissue.32

Mechanisms of hrsACE2 Construction and Administration

The hrsACE2 is administered by intravenous (IV) infusion twice daily for 5 minutes. It can help the pathological management of human disease by increasing Ang1-7, Ang1-8, and Ang1-9.33 Based on previous studies, administration of hrsACE2 was well tolerated with no apparent drug-related side effects. The copy number of SARS-CoV-2 decreased by 270 copies per mL with rapid plasma clearance.34
Mechanism of Rho-kinase Inhibitor Construction and Administration

Rho-kinase inhibitors encapsulated in aerosol liposomes have the potential to increase bioavailability and solubility. Using liposome nanoparticles as encapsulation could be a practical approach to producing vasodilation of the distal pulmonary arterioles, extending half-life, and increasing plasma concentration and absorption rate. Encapsulated drugs are administered as aerosols by inhalation via the pulmonary route to improve drug delivery to the lungs.

Pharmacokinetics of Rho-kinase inhibitor and hrsACE2 in COVID-19 patients

hrsACE2 was administered intravenously, with the concentration determined by calculating the ACE2 activity and content in the plasma sample. IV administration was preferred to avoid first-pass metabolism by the liver in oral administration. Administration of hrsACE2 was then continued for seven days and was well tolerated, accompanied by a decrease in the copy of SARS-CoV-2 without inhibiting the formation of anti-SARS-CoV-2 IgA and IgG antibodies. Furthermore, hrsACE2 has a half-life of 48 hours in the human body.

The distribution of injected hrsACE2 is uniform throughout the blood plasma. Metabolism of substrate Ang1-8 to product Ang1-7 prevents AngII-mediated ACE2 downregulation and shifts the angiotensin peptide balance in favor of AngII metabolism by ACE2. This will catalyze further production of Ang1-7, sustaining ACE2 transcription, membrane protein expression and eventually shedding that enhance ACE2 circulating activity.

Although the plasma concentrations were hrsACE2 dose-dependent, the biphasic terminal elimination half-lives were not (10.4±4.0 h (mean±SD)) (Table 1). The distribution, shown by Vd and Cmax, and the clearance showed no change in repeated administration in all different dosages (Table 1). Daily 100-1200 µg/kg administration of hrsACE2 for three consecutive days showed no significant accumulation with time, which is congruent with the ELISA finding of hrsACE2 elimination half-life (Table 1).

Rho kinase-inhibitor treatment is encapsulated with aerosol liposomes by inhalation administration. Liposomes themselves have been used for several years as drug delivery to antimicrobial agents for the lungs, various peptides, and drugs of small molecular weight to produce systemic effects. Research shows that liposome-encapsulated medications are safe for the lungs because the liposomes can control the way the drug is released, thereby reducing the amount of medicine that causes side effects. The inhalation mechanism was chosen because it can deliver medications directly to the lungs with a reduced dosing frequency. Clearance of liposomes is carried by the resident macrophage in the reticuloendothelial system (RES) through direct interaction with phagocytic cells whose absorption is due to vesicle opsonization. Unfortunately, liposomes have several disadvantages due to the enhanced permeability and retention (EPR) effect. This effect of EPR can be reduced by conjugating PEG on the liposomes so that the steric stabilization and circulation time modality will be increased.

Inhibition of SARS-CoV-2 infection by hrsACE2

The binding affinity between ACE2 and the receptor-binding domain (RBD) of the SARS-CoV-2 spike glycoprotein is 10 to 20 times higher than that of SARS-CoV RBD, possibly supporting higher pathogenesis SARS-CoV-2. hrsACE2 does not have a membrane anchor and circulates in small amounts in the blood. This soluble form can act as a competitive interceptor for SARS-CoV-2 by preventing the binding of viral particles to ACE2 receptors.

hrsACE2 minimizes lung injury, and multiple organ dysfunction competes for membrane-bound ACE2 and thus decreases the cell entry of SARS-CoV-2 into the target cells. Such hrsACE2 reduces viral growth of SARS-CoV-2 by a factor of 1000–5000 in cell culture, engineered human blood vessels, and kidney organoids. According to recent research, increased hrsACE2 at the tissue site can effectively compete with endogenous ACE2 and limit the entry of SARS-CoV-2 into host cells and lower AngII levels.

Increase ACE2 by Rho-kinase Inhibitor on ROCK Pathway

Inhibition of regulation of the RhoA-Rho kinase pathway responds by increasing the expression of AngII associated with improved regulation nitrate oxidation system with an increased endothelial expression of nitric oxide synthase subunits. Upregulation of the nitrate oxidation system on Rho-kinase inhibition can provide a protective effect against cardiovascular disease. Rho-kinase inhibitors have also been reported to activate the Akt pathway, which induces an increase in the expression of heme oxygenase-1, an anti-apoptotic and antioxidant enzyme. Inhibition of regulation of the RhoA-pathway
Rho kinase is also accompanied by reduced mRNA and AngII-induced protein expression p115RhoGEF and p63RhoGEF. Moreover, ACE2, carboxypeptidase A, and prolyl carboxypeptidase can produce Ang1-7 and AngII.66–50

Rho-kinase Inhibitor Inhibits the Formation of Microvesicles

RhoA/ROCK signaling plays a vital role in microvesicle formation. The study of Y27632, the competitive inhibitor ROCK1 and ROCK2, was able to prevent the thrombin-induced release of microvesicles. Further research on the use of rhinokine inhibitors on hCMEC/D3 cells led to decreased clearance of microvesicles in response to tumor necrosis factor (TNF) and changes in cell surface morphology. The activity also correlates with the action of proteolytic enzymes such as Stathmin and Calpain, which make the cell membrane unstable.51,52

Clinical Effects of hrsACE2 in the Treatment of COVID-19 Patients

Research conducted by Zoufaly et al. showed that attachment of hrsACE2 to the protein S could divert the penetration of the SARS-CoV-2 virus with the ACE2 receptor. A study by Monteil, et al. tested hrsACE2 on human capillary organoids and human kidney organoids, which also had a good effect on the inhibition of SARS-CoV-2 infection and tissue protectives in organoids.29,32,33,53,54

The plausibility of Rho-kinase inhibitors is demonstrated by the inhibitory ability of coagulation in lung tissue mediated by fibrin accumulation. The other clinical effects of Rho-kinase inhibitors in COVID-19 patients are summarized in Table 3 with a focus on past trials on cases of associated pulmonary disease.31,55,56

Clinical Effects of Rho-kinase inhibitors on ACE2 in the treatment of pulmonary disease in COVID-19 patients

Abedi et al., suggested that Rho-kinase inhibitors play an essential role in activating ACE2 and its levels. Furthermore, an in vivo study using mice by Xu et al., found that Rho-kinase inhibitors, Y27632 and HA1077, could block upregulated ACE and ACE2 in the overexpression of Rho-kinase. A downregulation of ACE2 mRNA by 65% and a decrease in ACE by 2.5 times were found in ACE2 dysregulation contributing to acute pulmonary embolism.42

The same thing happened in upregulated ACE and AngII, significantly attenuated by Rho-kinase inhibitors Y27632 and HA1077. In the same study, downregulation of ACE2 and Ang1-7 was also significantly inhibited by Rho-kinase inhibitors.42

Conclusion

COVID-19, a disease caused by SARS-CoV-2, has become a global health problem. Various therapeutic approaches to prevent and treat the disease have been made from multiple aspects. Ethanol extraction and compounds have the potential to become Rho-kinase inhibitors which can be packaged using liposome drug delivery and administered by inhalation. This inhibitory agent can inhibit microvesicle formation and regulate ACE2 regulation. ACE2 overexpression in most COVID-19 patients and the use of ACE inhibitors, one of which is hrsACE2, can bind to viral proteins so that it can minimize injury to many organs.

Conflict of Interest

None

References

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