Molecular Docking Human Plasma Kallikrein to Prevent Acute Respiratory Distress Syndrome(ARDS) in COVID-19 Patient

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Abstract

SARS CoV-2 infection causes various clinical manifestations ranging from mild to severe. Acute Respiratory Distress Syndrome (ARDS) is a severe complication of COVID-19 caused by activation of the kallikrein-kinin system which produces bradykinin which is a potent proinflammatory mediator. This research is an in silico study which aims to determine the potential of active medicinal plant compounds in inhibiting the kallikrein-kinin system.Molecular docking in this study using Autodock 4.2 with Lamarckian GA criteria. Human plasma kallikrein (PDB ID: 5TJX) was docked with 70 compounds and one native ligandand analyzed using Autodock 4.2.The smallest binding energy obtained from docking 5TJX with several compounds in sequence, namely, xanthohumol, nafamostat, demethoxycurcumin, epicatechingallate, beta mangostin, alpha mangostin (-9.52, -9.35, -9.33, -9.28, -9.19, -9.06 kcal/mol). Therefore, the compound shows the best potential as a plasma kallikrein inhibitor. However, further research is still needed to determine the potential of drugs and medicinal plant active compounds for medical treatment.

Keyword: *COVID-19, ARDS, Kallikrein, Medicinal Plant, Docking, Health Risk*

Introduction

SARS Cov-2 infection causes various clinical manifestations, from asymptomatic, mild, or moderate symptoms, to severe cases requiring intensive care [1]. In severe cases, one of the serious complications that often occurs is Acute Respiratory Distress Syndrome (ARDS). Several studies from Wuhan, China reported the incidence of ARDS in COVID-19 patients of 14-29%, and among critically ill patients around 67%. ARDS is caused by extensive endothelial-barrier damage and an uncontrolled 'cytokine storm'. Increased levels of cytokines in SARS Cov-2 infection result in endothelial dysfunction, deregulation of coagulation, and increased microvascular permeability, leading to tissue edema and shock that can develop into acute lung injury (ALI) and ARDS [2].

The Kallikrein-kinin system mechanism can also contribute to lung injury and angioedema which can lead to ARDS. Kallikrein is a serine-protease enzyme that converts kininogens into kinin plasma protein.

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Dysregulation of the Kallikrein-kinin system in COVID-19 patients potentially affected by decreased ACE2 expression due to SARS Cov-2 infection [3]. SARS Cov-2 also expresses cysteine proteases which can activate the kinin pathway by interacting with kininogens. Activation of the Kallikrein-kinin system causes the release of bradykinin. Bradykinin-receptor B2 (BKB2R) contributes to the pro-inflammatory pathway by activating nitric oxide (NO) and prostaglandins (PGs) thereby increasing vascular permeability [3,4]. Bradykinin can contribute to lung injury and inflammation in patients with COVID-19 [5].

The development of natural compounds that may have similar therapeutic potential with smaller adverse effects is considered to be used as therapeutic alternatives or as complementary therapies to current ALI therapies. Natural compounds can also form the basis of new drugs for the treatment of diseases[6]. In recent decades, various natural compounds extracted from plants have been reported to have anti-inflammatory activity.Several polyphenolic compounds have shown important in vivo and in vitro anti-inflammatory activity, such as flavonoids, lignans, phloroglucinols, quinones, stilbenes, phenylpropanoids, and diarylheptanoids. Also, terpenoid compounds have shown anti-inflammatory effects[7].

Kallikrein-kinin system inhibitor is one of the ways to minimize widespread inflammation due to SARS Cov-2 infection, so it is supposed to reduce the incidence of ALI and ARDS in critically ill COVID-19 patients.Currently, there has not been much research on natural compounds that have the potential to inhibit the Kallikrein-kinin system. In this study, the authors conducted a molecular docking study using human plasma kallikrein (PDB ID: 5TJX) as a protein target and several natural compounds which aimed to determine the potential of these active compounds in inhibiting the formation of bradykinin.

Material and Methods

Protein Receptors Preparation

The 3D structure of human plasma kallikrein (PDB ID: 5TJX) was downloaded from the Protein Data Bank (https://www.rcsb.org/) in .pdb format[8]. The active site of the protein was determined using Discovery studio 2016[9]. Protein optimization by removing water, adding polar hydrogen, and adding charge using Autodock 4.2. The structure of the protein was saved in .pdb format for further analysis.

Ligand Preparation and Drug Likeness Activity

The 3D structure of each natural compound was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in .sdf format and being optimized using Avogadro 1.2 and converted format into .pdbqt by using Open Babel 2.4. The natural compound with anti-inflammatory effect was obtained from Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search).

The drug-likeness analysis was conducted to know the molecules with good permeation and oral absorption have molecular weight <500 Da, C log*P*<5, H-bond donor <5, H-acceptor <10, violation <2, this was calculated using the SWISSADME (http://www.swissadme.ch/)[10].

Molecular Docking

The device used in this study is a laptop with an Intel Core i5-7200U CPU @ 2.50-2.71GHz, 8GB RAM. Operating System using Windows 10 pro ver 1903, 64-bit OS. Firstly molecular docking was performed with the native ligand of the protein to validate the reliability of the molecular docking process with root mean square deviation (RMSD) value <2 Å. The grid coordinate (X,Y,Z) -11.377, -2,117, -16,39 with dimensions of the Grid Box 36x36x36. Throughout the docking process, the macromolecule was kept rigid and the ligand was flexible. The molecular docking was performed using Autodock 4.2 by adjusting the Genetic algorithm (GA) parameter, using 10 runs of the Lamarckian GA criteria. Human plasma kallikrein (PDB ID: 5TJX) was docked with 70 compounds and one native ligandand analyzed using Autodock 4.2.

Result and Discussion

In the 5TJX protein, there is only one native ligand, namely GBT with active sites LEU418, HIS434, HIS472, GLN473, SER478, GLU479, GLY480, TYR555, ARG560, MET561, ASP572, ALA573, CYS574, LYS575, GLY5975, SER578, THY599, CYS602. Several drug candidate compounds have been selected according to the Lipinski Rule of Five (Table 1)then sorted according to their binding energy. The smallest binding energy obtained from docking 5TJX with 70 compounds in sequence, namely, xanthohumol, nafamostat, demethoxycurcumin, epicatechingallate, beta mangostin, alpha mangostin, avoralstat, camostat (-9.52, -9.35, -9.33, -9.28, -9.19, -9.06, -8.41, -8.23 kcal/mol).

Table1. Lipinski Rule of Five Properties of Human Plasma KallikreinPotential Inhibitors

No	Compound (Molecular Formula)	Lipinski's rule of five				
		Molecular weight (<500 Da)	LogP (<5)	H-Bond donor (<5)	H-bond acceptor (<10)	Violation
1	Xanthohumol (C21H22O5)	354.40	3.76	3	5	0
2	Nafamostat (C19H17N5O2)	347.37	2.11	4	4	0
3	Avoralstat (C28H27N5O5C30H26F4N6O)	513.54	2.53	5	7	1
4	Camostat (C21H26N4O8S)	494.52	1.30	3	9	1
5	Demethoxycurcumin (C20H18O5)	338.35	3.00	2	5	0
6	Epicatechin gallate (C22H18O10)	442.37	1.23	7	10	1
7	Beta mangostin (C25H28O6)	424.49	4.97	2	6	0
8	Alpha mangostin (C24H26O6)	410.46	4.64	3	6	0

Kallikrein is a serine protease enzyme that converts kininogens into kinin plasma protein. Activation of kallikrein causes the release of bradykinin which contributes to the pro-inflammatory pathway. The kallikrein-kinin system can be a factor that plays a role in the formation of lung injury and angioedema in COVID-19 patients [3]. This study used human plasma kallikrein (PDB ID 5TJX) as a potential protein target for the prevention of ARDS in critically ill COVID-19 patients. The ligands that will be tested are berotralstat, avoralstat, nafamostatmesylate, camostatmesylate, and several natural compounds that have anti-inflammatory effects to assess potency as a plasma kallikrein inhibitor.

In this molecular docking study, we used berotralstat, avoralstat, nafamostat, and camostat as standard drugs for comparison.Berotralstat is a specific plasma kallikrein inhibitor therapy given orally once-

daily dose. Berotralstat has completed its phase III study as long-term prophylaxis for Hereditary Angioedema due to deficiency of C1-inhibitor (C1-INH-HAE). Berotralstat was reported to reduce attacks of C1-INH-HAE angioedema in a phase III trial [11,12]. Avoralstat is a potent plasma kallikrein inhibitor. Oral avoralstat therapy has been tested in phase III trials to shorten angioedema episodes and improve the quality of life of patients with C1-INH-HAE compared to placebo therapy [13].

Nafamostatmesylate and camostatmesylate are synthetic serine protease inhibitors. Nafamostat inhibits several enzyme systems, namely the coagulation and fibrinolytic systems (thrombin, Xa, and XIIa), the kallikrein-kinin system, the complement system, pancreatic proteases, and protease-activated receptor (PARs) activation. In COVID 19, Nafamostat may prevent disease progression by controlling the immune system such as the complement cascade, preventing disseminated intravascular coagulation (DIC), and preventing viral invasion by inhibiting viral fusion in cell membranes [14,15]. Camostat inhibits cholecystokinin, proinflammatory cytokines, and serine proteases [16]. Camostat is used for the treatment of chronic pancreatitis, drug-induced lung injury, and has the potential as COVID 19 therapy by preventing the entry of the virus into lung cells [17].

The results of docking between human plasma kallikrein (PDB ID: 5TJX) and ligands using Autodock 4.2 showed binding energy ranges of -9.35 and -3.59.The natural compound that has the smallest binding energy is xanthohumol with -9.52.Nafamostat, Avoralstat, Camostat, and Berotralstat have binding energies of-9.35, -8.41, -8.23, and -3.59 respectively. Xanthohumol is a prenylflavonoid found in the hop plant (Humuluslupulus L.) which is used in brewing and medicine.In the previous study, Xanthohumol could downregulate inflammatory mediators by inhibiting transactivation of NF-κB in LPS-activated macrophages and inhibiting STAT-1α and IRF-1 activation in IFN-activated macrophages [18]. Xanthohumol could effectively inhibit LPS-induced oxidative stress and inflammatory damage of lungs, which may be associated with upregulation of the Nrf2 pathway depending on AMPK activation and GSK3β inhibition [19].

Demethoxycurcumin has a binding energy of -9.33. Demethoxycurcumin can be found in turmeric (Curcuma longa) and ginger (Curcuma xanthorriza). This compound has anti-inflammatory, antioxidant, and antiproliferative effects.Demethoxycurcumin significantly reduces inflammation due to increased expression of IL-1 β, IL-6, NF-κB, TNF-α, iNOS, and COX-2 [20]. Epicatechingallate exhibits binding energy of -9.28. Epicatechingallate is mostly found in the tea plant (Camellia sinensis).Epicatechingallate has been studied to have anti-inflammatory effects, which reduce the expression of TNF-α, IL-1β thereby attenuating the expression of iNOS and COX-2 [21]. Beta mangostin has a binding energy of -9.19. Beta mangostin can be isolated from hexane and chloroform extracts of mangosteen stem bark (Garcinia mangostana).Beta mangostin has anti-inflammatory effects by inhibiting the production of nitric oxide (NO) [22]. Based on the binding energy from the docking result, xanthohumol, demethoxycurcumin, epicatechingallate, beta mangostin, and alpha mangostin, are natural compounds that are recommended as potential inhibitors of plasma kallikrein for further in vitro and in vivo studies.

Conclusion

COVID-19 has become a global health problem due to the high spread and until now there has been no adequate therapy for ARDS complications which are triggered by activation of the kallikrein-kinin system which produces potent pro-inflammatory mediators. Therefore we recommend xanthohumol, nafamostat, demethoxycurcumin, epicatechin gallate, beta mangostin, alpha mangostin which show the best potency as plasma kallikrein inhibitors. However, further research is still needed to determine the potential of drugs and medicinal plant active compounds for medical treatment.

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