73696_Auto_Edited.docx

WORD COUNT 4531
TIME SUBMITTED 17-MAR-2022 08:17PM
PAPER ID 83766595

Name of Journal: *World Journal of Hepatology*
Manuscript NO: 73696
Manuscript Type: ORIGINAL ARTICLE

Basic Study

COVID-19 Liver and gastroenterology findings: A in silico analysis of SARS-CoV-2 interactions with liver molecules

Gabrielle Caroline Peiter, Cristiano de Bem Torquato de Souza, Lucca Miketen de Oliveira, Luis Gustavo Pagliarin, Valentina Nunes Fontoura dos Anjos, Filipe Antônio França da Silva, Fabrício Freire de Melo, Kádima Nayara Teixeira

Abstract

BACKGROUND

Coronavirus disease 19 (COVID-19) not only has shown to affect the respiratory system, but also has demonstrated variable clinical presentation including gastrointestinal tract disorders. Besides that, abnormalities in liver enzymes were reported indicating hepatic injury. It is known that SARS-CoV-2 might infect cells through the viral receptor Angiotensin-Converting Enzyme 2 (ACE2) which is expressed in several organs including the liver. The viral Spike glycoprotein binds to the ACE2 and must be cleaved by Furin and TMPRSS2 proteases to get into the cells. After that, the Akt/mTOR signaling pathway is activated and several COVID-19 changes are triggered.

AIM

To analyze liver and gastrointestinal symptoms and cell signaling pathways triggered by SARS-CoV-2 infection due to virus-liver interactions *in silico.*

METHODS

In this *in silico* study the three-dimensional structures of the Akt, mTORC1 and Furin (receptors) were selected from the Protein Data Bank (PDB) and the structures of inhibitors (ligands) MK-2206, CC-223 and Nafthofluorescein were selected from PubChem and ZINC databases. Ligand files were downloaded as 2D structures and they were converted to optimizing 3D structure using ViewerLite 4.2 software. Marvin Sketch® software was used to calculate the prediction of protonated form of inhibitors at physiological environment (pH 7.4). AutoDock Tools (ADT) was used to calculate and delimit the Grid box used in the molecular docking of each structure selected in the PDB. In addition protonated ligands were prepared for molecular docking using ADT software. Molecular docking was performed using ADT software tools connected to Vina software. Analysis of the aminoacid residues involved in ligand interactions, as well as ligand twists, the atoms involved in interactions, bond type and strength of

interactions were performed using PyMol® and Discovery Studio® (BIOVIA) softwares.

RESULTS

Molecular docking analysis shows that mTORC1/CC-223 complex has affinity energy between receptor and ligand of -7.7 kcal/mol with interactions ranging from 2.7 to 4.99 Å. There are four significant chemical bonds which involve two out five polypeptide chains that form the FRB (FKBP12–Rapamycin-Binding) domain. The strongest one is a Hydrogen bond - the only polar interaction, and Van der Waals interactions show to be present in 12 residues of mTORC1's FRB domain. Regarding the Akt/MK-2206 complex there are three Van der Waals interactions and 12 chemical bonds in which seven residues of Akt are involved with all five rings of the MK-2206 structure. In this way, both ASP 388 and GLN 391 bind to the same MK-2206 ring, the smaller one. Yet, LYS 386 makes four chemical bonds with the inhibitor, one with each structure ring, while LYS 387 binds to two distinct rings. One of the MK-2206 inhibitor's rings which binds to LYS 387 also binds simultaneously to ILE 367 and LEU 385 residues, and the fifth ring of the structure is involved in a bond with the ALA 382 residue. The Hydrogen bonds are the shortest ones in the complex (2.61 and 3.08 Å) and all interactions confer an affinity energy of - 8.8 kcal/mol. The affinity energy in the Furin/Nafthofluorescein complex is -9.8 kcal/mol and involves six interactions ranging from 2.57 to 4.98 Å. Among them, two are polar and the others are non-polar, in addition to twelve more Van der Waals interactions. Two distinct Hydrogen bonds were formed between Furin and its inhibitor involving GLN 388 and ALA 532 residues. ALA 532 also binds to two distincts rings of the Nafthofluorescein while TRP 531 residue makes two simultaneous bonds with the inhibitor.

CONCLUSION

Liver infection and signaling pathways altered by SARS-CoV-2 can be modulated by inhibitors that demonstrate significant interaction affinity with human proteins, which could prevent the development of infection and symptoms.

Key Words: Bioinformatic; Cell signaling pathway; COVID-19; Liver injury; SARS-CoV-2

Peiter GC, de Souza CBT, de Oliveira LM, Pagliarin LG, dos Anjos VNF, Silva FAFD, Freire de Melo F, Teixeira KN. COVID-19 Liver and gastroenterology findings: A in silico analysis of SARS-CoV-2 interactions with liver molecules. *World J Hepatol* 2022; In press

Core Tip: The COVID-19 pandemic has led to profound changes in the world scenario in relation to health and scientific research by seeking effective treatment and mitigating the number of cases and deaths. This type of intervention requires molecular level basementing to understand what is actually affected by the virus and thus understand the development and worsening of the infection and the appearance of symptoms. The emergence of COVID-19 as a type of respiratory disease, based on the most severe clinical symptoms of the first cases of the disease, caused the focus of the studies to be directed in this sense. Thus, mild clinical symptoms, such as gastrointestinal symptoms, have been few studied. Currently, with the knowledge that COVID-19 is a systemic disease, further studies on liver damage become important. In this study we analyzed liver molecules that are the target of SARS-CoV-2 infection through inhibition studies in silico. As much as such molecules are present in several other organs, due to the central role of the liver in systemic metabolism, trying to understand metabolic changes in this organ will help understand systemic changes.

INTRODUCTION

At the end of 2019, in Wuhan (China), the emergency of a new coronavirus (SARS-CoV-2) was reported, which is classified as belonging to the *Beta-coranavirus* genus and possessing as genetic material a single strip positive RNA, being capable of resulting in disease (COVID-19) that can evolve to severe acute respiratory syndrome. Although it has been known that this disease affects mostly the respiratory system, it was identified that it can manifest clinic signs and symptoms related to other organs, such as nausea, vomit, abdominal pain and diarrhea. Concerning the liver, alterations in lesion markers including Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and total serum bilirubin was reported, indicating hepatic injury [1,2]. Between 14%-53% of patients with COVID-19 present the referred abnormalities in transaminase levels[3,4] which is associated with severity of the disease, abnormal transaminase levels can indicate higher chance of bad prognosis and Intensive Care Unit requirement[5-7]. The associated causes for liver injury, beyond direct viral impact, includes the use of drugs during treatment, hypoxia due to pulmonary symptoms, previous hepatic lesions and co-morbidities[4]. Some of the drugs used can cause hepatotoxicity, liver injury and dysregulation, that's the case for hydroxychloroquine, Azithromycin and Remdesivir, respectively[8-10]. One of the SARS-CoV-2 presentations on this system can be as acute non-icteric hepatitis before the most common symptoms (fever and respiratory symptoms)[11] and those already related to the liver, nausea, diarrhea and abdominal pain. The viral cellular entrance and its dissemination on a vast spectrum of the organism systems, for example the liver-related, is a consequence of the expression of a cellular common receptor (Angiotensin-Converting Enzyme 2 or ACE2). However, for the effective cellular intrusion it is requested the participation of other human proteins as TMPRSS2 (Type 2 Serine Protease) and Furin (Convertase Proprotein of the Subtilisin Type) [1,2,12-15]. Therefore, concerning the entrance mechanisms, has been identified that the viral Spike glycoprotein possess affinity and binds to the ACE2 (responsible for the adhesion stage), bringing to mind that the last is structurally divided in two subunits: S1 (N-terminus, that connects to the receptor) and S2 (C-terminus, that take a part in the penetration process) [2,13,14]. Between both units, S1 and S2, there is a cleavage site for

Furin, that triggers the activation and conformational change of viral Spike glycoprotein after it completes action [2,14,15]. Following the initial activation process, another cleavage between the S1/S2 and S2' sites is essential for the operation of viral entrance. On this, the TMPRSS2 protein performs the referred activity over the Spike glycoprotein of the SARS-CoV-2, which allows the fusion between the viral and cellular membranes, entry of the viral genetic material and the development of the infection [1,12,13]. With the viral entrance, during the course of infection, occurs modulation of the signaling pathway Akt/mTOR, that regulates apoptosis, cell survival, transcription and translation, which also occurs during infection by other viruses [16-18]. This signaling possibly increases factors of viral translation while blocking mechanisms of cellular death, generating a greater pathogenicity. Based on this, recent studies indicate a possibility of use of already existing drugs that interfere in this pathway for the treatment of COVID-19, among those is the MK-2206, an Akt inhibitor [19].

Despite those facts, studies directly abording the interaction between SARS-CoV-2 and the liver cells, specifying the entrance mechanisms, the passage by the biochemical cascades and methods for possible infection inhibition still need investigation. Based on the previous explanation, this study aims to associate the hepatic alterations triggered by SARS-CoV-2 with the activation and/or inhibition of transduction pathways of cellular signals in the viral infection process. Besides, some possibilities of intervention in the signaling pathways with inhibitors were analyzed to suggest possible treatments for SARS-CoV-2 infection.

MATERIALS AND METHODS

Receptors and ligands preparation

Akt, mTORC1 and Furin enzymes are inhibited by MK-2206, CC-223 and Nafthofluorescein, respectively. The three-dimensional structures of proteins/enzymes (receptors) Akt, mTORC1 and Furin were selected from Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do) and their files were obtained in the extension .pdb (input file). The structures of the inhibitors drugs (ligands) MK-2206,

CC-223 and Nafthofluorescein were selected from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and ZINC databases (https://zinc12.docking.org/). The ligands' files were downloaded as 2D structures in the extension .sdf and they were converted into .pdb (input file) by optimising the 3D structure using ViewerLite 4.2 software (Accelrys Inc.). The selected receptor's three-dimensional structures were prepared for molecular docking simulations using AutoDock Tools (ADT) software (MGL, The Scripps Research Institute); the possible ligands (ions, peptides) were eliminated, the water molecules were deleted and hydrogen atoms were added. Then it was determined through the ADT software the location and dimensions of the Grid box (virtual box that delimits the region where the ligand will perform possible interactions with the receptor). The Grid box data and coordinates were used in molecular docking. The ligands interact with their receptors in a physiological environment with pH 7.4, thus, to simulate more reliably their interaction was made the calculation for the prediction of its protonation state using the Marvin Sketch® software (ChemAxon®). Protonated ligands were prepared for molecular docking using the ADT software. This software detects the torsion points of the inhibitors and calculates their angle of the torsions.

Molecular docking

Molecular docking procedures for a rigid receptor and a flexible ligand were used. A grid of points in x, y, and z directions was built with a grid spacing of 1.0 Å using the AutoGrid component of the software. Molecular docking simulations were performed using the tools of the ADT software connected to Vina software [20]. The software used associates two components: a search algorithm and a score function. The algorithm is responsible for the search of possible combinations in the bonds, exploring the rotational, translational and conformational degrees of freedom of the ligand, as well as of the proteins. Then, the score function is used to choose the best binding modes. These functions are obtained according to the force fields of molecular mechanics and empirical parameters from free energy calculations, thus an affinity energy is

calculated. The cut-off for stable interactions is considered an affinity energy < −6.0 kcal/mol [21]. The results are based on the first docking conformer of the ligands with reference Root-Mean-Square Deviation of atomic positions (RMSD) of 0 [22]. The analyses of the amino acid residues of receptors involved in the interactions with the ligands, as well as the twists of the ligand, atoms involved in the interactions, type, strength and length of the interactions were performed using the PyMol® software (pymol.org/2) and Discovery Studio® software from BIOVIA.

RESULTS

Receptors and ligands structures

The receptor's 3D structures selected were 5WBH - FRB domain of mTOR; 1GZN - PKB kinase domain and 5JXI - Furin convertase. All structures are human and present less than 2.5 Å of resolution. Inhibitor' structure MK-2206 was selected from ZINC databank - ZINC36382821 and inhibitors' structures CC-223 (CID58298316), Nafthofluorescin (CID3124834) were selected from PubChem databank.

Interactions of protein-inhibitor complexes

mTORC1/CC-223 complex

Molecular docking was used to analyze if there is an interaction between mTORC1 enzyme and CC-223 in its protonated form. In this way, the most stable mTORC1/CC-223 complex showed an affinity energy of -7.7 kcal/mol (Figure 1). Four significant chemical bonds were observed between the receptor and the ligand involving four distinct residues and bond size ranging from 2.7 to 4.99 Å (Table 1). Of these bonds only one is polar (Hydrogen bond) and according to their size, this is the strongest one (2.7 Å); the other bonds have apolar character. In addition, 12 other residues are involved in Van der Waals interactions (Figure 2). The four significant bonds in the mTORC1/CC-223 complex involve two (C and D) out five polypeptide chains (A-E) that form the FRB (FKBP12–Rapamycin-Binding) domain.

Akt/MK-2206 complex

Analysis of the interaction between the Akt enzyme and its protonated inhibitor - MK-2206, showed that the complex is maintained by 12 chemical bonds, in addition to three Van der Waals interactions (Figure 3 and Table 1) . All five rings of the MK-2206 structure are involved in bonds with amino acid residues of Akt enzyme. Seven residues of Akt are involved in chemical bonds; ASP 388 and GLN 381 residues bind to an oxygen atom and a nitrogen atom of MK-2206 by hydrogen bond, respectively. Hydrogen bonds are the shortest of the Akt/MK-2206 complex (2.61 and 3.08 Å). ASP 388 still binds to MK-2206 by Pi-Alkyl bond (hydrophobic character). Both ASP 388 and GLN 391 bind to the same MK-2206 ring, the smaller one. LYS 386 makes four chemical bonds with the inhibitor (3.08 - 5.25 Å) , one with each structure ring; three of them are Pi - Cation and another is Pi - Alkyl. LYS 387 binds to two distinct rings of the MK-2206 inhibitor by Pi- Alkyl bonds (5.19 and 5.22). One of the MK-2206 inhibitor's rings that binds to LYS 387 also binds simultaneously to two other residues - ILE 367 (Pi - Alkyl) and LEU 385 (Amide - Pi). The cited bonds involve four out five rings of MK-2206. The fifth ring of the structure is involved in a single Pi- Alkyl bond with the ALA 382 residue. All bonds and their distribution confer an affinity energy of - 8.8 kcal/mol (Figure 1).

Furin/Nafthofluorescein complex

Molecular docking was used to analyze if there is an interaction between Nafthofluorescein and human furin. In this way, the simulation helps to better understand the interaction dynamics between the protein and the inhibitor. After analyzing the protonation state of Nafthofluorescein, the interaction affinity was calculated by AutoDock Vina, and was -9.8 kcal/mol for the complex formed. It was possible to observe that Nafthofluorescein interacts with human Furin through six interactions with a length ranging from 2.57 to 4.98 Å. Two distinct hydrogen bonds were formed between Furin and its inhibitor involving GLN 388 and ALA 532 residues. Among them, two are polar and the others are non-polar, in addition to twelve more

Van der Waals interactions. ALA 532 also binds to two distincts rings of the Nafthofluorescein by Pi- Sigma bond and Pi - Alkyl bond. TRP 531 residue makes two simultaneous bonds with the inhibitor (Figure 4 and Table 1).

DISCUSSION

Liver is the main organ involved in drug metabolization and xenobiotic detoxification, so its proper functioning is essential to the effectiveness of pharmacological treatments. Since altered liver function is reported in up to half of the patients with COVID-19, it is important to clearly understand the possible mechanisms involved in liver injury in order to optimize the outcome in the treatment of this disease[23].

Moderate microvesicular steatosis and mild inflammation in the lobular and portal area are pathological findings in liver tissue in patients with COVID-19[24], so, this may contribute to the incidence of elevated levels of hepatic transaminases reported in this disease[25]. In the early stage of COVID-19, infected individuals had positive SARS-CoV-2 RNA in fecal and blood samples, and present gastrointestinal symptoms such as diarrhea, abdominal pain, nausea, and vomiting[26] suggesting that SARS-CoV-2 could infect liver cells[27].

Regarding SARS-CoV-2 infection, it is known that the viral Spike glycoprotein interacts with the ACE2 present in humans, leading to the entry of the vírus. However, for its entry to occur, there is a need for activation of the glycoprotein by host cell proteases which occurs between the S1/S2 subunits of Spike generating the conformational change of the S2 subunit and allowing the interaction of SARS-CoV-2 with ACE2, completing the entry[13].

Since SARS-CoV-2 interacts with the ACE2 receptor of the host cells to invade them[28], thus, cells that have this receptor are susceptible to infection. The level of ACE2 expression is low in hepatocytes (2.6%), but in bile duct cells (cholangiocytes) this expression is high (59.7%)[29]. Therefore, SARS-CoV-2 does not necessarily directly infect liver cells, but causes bile duct dysfunction that plays an important role in liver regeneration and immune response[27].

In fact, according to Xu *et al* (2020), no direct cytopathic effect of SARS-CoV-2 on the liver was found in the pathological autopsy findings. On the other hand, Pirola and Sookoian[30] findings support the possibility that SARS-CoV-2 may cause direct liver injury by viral cytopathic effect. These authors showed that the three host cell proteins - ACE2, Furin and TMPRSS2, responsible for viral infection are expressed in liver tissue. Although ACE2 has low expression in hepatocytes compared to cholangiocytes, TMPRSS2[31] and Furin[32] are more expressed in hepatocytes.

The Spike glycoprotein of SARS-CoV-2 facilitates viral entry into host cells; the surface unit S1 binds to a cellular receptor - ACE2, while the transmembrane unit S2 facilitates fusion of both viral and cellular membrane. Membrane fusion depends on S protein cleavage by host cell proteases at the S1/S2 and the S2' sites[33], among them, TMPRSS2 and Furin play major roles in proteolytic activation of a broad range of viruses[34] including SARS-CoV-2. After infection, the signaling pathways of the host cell are affected to promote viral replication. Activation of the Akt/mTOR signaling and through a cascade of events, mTORC1 and Akt activate host transcription and translation of specific genes. The activation of Akt/mTOR signaling during SARS-CoV-2 infection could be to sustain protein synthesis by increased accession to translation components[35].

An analysis of the summary of the SARS-CoV-2 infection process indicates that host cell proteases and signaling cascade proteins could be a potential target for therapeutic interventions for COVID-19 symptoms, including gastrointestinal symptoms due to liver damage.

This study analyzed some interactions of SARS-CoV-2 with molecules which are necessary for the success of the infection that are expressed in the liver using bioinformatics tools and *in silico* enzymatic inhibition tests to try to associate such interactions with the gastrointestinal findings and liver injuries in COVID-19. It was analyzed the interaction affinity of some proteins present in the human body with their respective inhibitors that can act in their pathways and prevent the development of an

infection. The target proteins were the Furin enzyme, involved in cell invasion, and mTORC1 and Akt enzymes belonging to the signaling pathway.

To verify at the molecular level the interaction between the mTORC1/CC-223, Akt/MK-2206 and Furin/Nafthofluorescein complexes, molecular docking was used. Thus, if there is an interaction between proteins and inhibitors, simulation helps us to understand the dynamics that happen *in silico*[36].

The interaction affinity calculated by AutoDock Vina for mTORC1/CC-223 complex was -7.7 kcal/mol, for the Akt/MK-2206 complex it was -8.8, whereas for the complex formed by Furin/Nafthofluorescein it was of -9.8 kcal/mol. These values are considered significant since values lower than -6.0 kcal/mol already constitute stable interactions *in silico* analysis[37].

The complex with the highest interaction affinity is that formed by Furin and its inhibitor. This significantly low affinity energy value indicates a more stable complex, in other words, indicates that the inhibitor will have higher biological activity[38]. Unlike other coronaviruses, the SARS-CoV-2 has a potentially critical insertion of a Furin cleavage site upstream of the S1 cleavage site in spike reducing its dependence on host cell proteases for infection. The high affinity between ACE2 and the Spike glycoprotein cleaved by Furin allows SARS-CoV-2 to maintain its efficient entry into cells while preventing the action of the immune system which can contribute to the widespread infection capacity of the virus[15,39]. Since ACE2 is present in type 2 alveolar cells, the gastrointestinal tract and the liver, these tissues would be more affected by COVID-19[40], so inhibiting the action of Furin would prevent infection of these tissues and consequently the associated symptoms.

Vankadari[41] in his study about Furin analyzed not only its structure but also how it would bind at S1/S2 subunits of Spike glycoprotein. Thus, it was suggested that Furin binds to these subunits through the equatorial region present in the Spike glycoprotein which creates a ~970 Å interface between the participants.

The Furin enzyme is required in various normal functions of the body[42]. Prolonged Furin blocking can therefore generate side effects or damage[43]. In this context, Furin's

involvement in the viral invasion process could reduce the effectiveness of the action of this enzyme in normal physiological processes triggering pathological processes. In fact, studies suggest that Furin plays an important role in homeostasis and disease[44], thus it is possible that liver cell lesions would be reduced and AST and ALT levels would be normal. On the other hand, a brief Furin inhibition can be well tolerated and offer a therapeutic benefit[43], so the Nafthofluorescein has a promising potential for treatment through this route given its high affinity for Furin *in silico.*

Another way to study the symptoms resulting from liver injury triggered by SARS-CoV-2 infection is to analyze the signaling pathway affected by the virus. In fact, some studies point to the dysregulation in Akt/mTOR signaling cascade, which could be a potential target for COVID-19 treatment[35].

It could be observed in this study that the Akt/MK-2206 complex remains with 12 bonds, added to three Van der Waals interactions and all five rings of the MK-2206 inhibitor are bound to protein, suggesting a stability formed in the complex, with it, the protein Akt would be unable to proceed with his cascade. Shi *et al.*[45] in his findings on the inhibition of esophageal cancer growth through the PI3K/AKT/mTOR pathway, he showed that MK-2206 would be a potential allosteric inhibitor of Akt, acting in decreasing cell proliferation, inducing cell cycle arrest and increasing apoptosis of cancer cells. Furthermore, Appelberg *et al*[35] observed that Akt/mTOR/HIF-1 pathways participate in COVID-19 infection, in particular, Akt/mTOR are activated at the beginning of the infection. Within this, it has also been shown that MK-2206 caused a decay in viral transcription in SARS-CoV-2 infected cells and supernatants by interacting with Akt. Based on these data and on the results obtained with molecular docking, in which a significant affinity and stability of the bonds was observed, it can be stated that the MK-2206 inhibitor has the possibility of helping to contain the development of COVID-19 to interact with Akt, in a way that prevents the continuity of the cascade triggered by this protein.

It is possible to note that in the mTORC1/CC-223 complex significant chemical bonds are established along the CC-223 structure involving three out of four molecule's rings.

The same is observed about Van der Waals' interactions. The sum of all binds and interactions between mTORC1 and CC-223 contribute to the formation of a complex with significant affinity energy which contributes to its stability. Mortensen *et al* showed CC-223 as an inhibitor that has high affinity for mTOR, so that the pathway to which it belongs was unfeasible. It has been described that mTOR is relevant in cell growth, proliferation, survival and metabolism; in particular, mTORC-1 plays a role in protein synthesis and cell development. In view of this, CC-223 has been reported to cause inhibition of mTORC-1 in vivo upon administration of this compound in tumor-bearing mice, suppressing the continuation of the cascade[46]. Added to this, in another study, Mortensen *et al* also analyzed the interaction of compounds that could interact with the PI3K/AKT/mTOR pathway, thus, the selectivity of the CC-223 inhibitor for the mTORC-1 protein was highlighted, since the former has a high affinity for the latter and may inhibit it[47]. . Furthermore, in line with the results obtained through molecular docking, it was found that the binding of the mTORC-1 and CC-223 complex has a high affinity and stability, therefore, considering the potential of this inhibitor for blockade of the mTOR pathway, it can be inferred that there is a possibility of using it to prevent the spread of SARS-CoV-2 infection and, consequently, restrict the disease spread. In this way, since the liver is the main regulatory body metabolism and the mTOR/Akt signaling pathway plays a key role in cellular metabolism, changes in this pathway significantly reflect on the liver; so, preventing changes on pathway by the virus would help to slow down the symptoms.

CONCLUSION

The current understanding suggests that the possible interaction between SARS-CoV-2 and liver cells occurs with enhancer factors that facilitate ACE2-mediated SARS-CoV-2 infection, like Furin and TMPRSS2. In this study, we demonstrate by means of molecular docking a significant affinity and stability of the bonds concerning some human hepatic proteins and their inhibitors. The last ones work in signaling pathways by interactions between mTORC1/CC-223, Akt/MK-2206 and Furin/Nafthofluorescein

complexes, being capable of preventing infection development. Hereby, our *in silico* analysis shows possible inhibitory mechanisms of cell viral reception in the liver, which is consistent with other studies. Therefore, multi-target therapeutic drugs based on these pathways may be an option to COVID-19 patients, especially in severe/critical ones, preventing multiple organ dysfunction and perhaps leading to more positive prognosis.

ARTICLE HIGHLIGHTS

Research background

COVID-19 has variable clinical manifestations, including gastrointestinal and hepatic disorders. SARS-CoV-2 infect liver cells using ACE2, and viral Spike must be cleaved by Furin or TMPRSS2. After, the Akt/mTOR pathway is activated and several changes are triggered.

Research motivation

Liver damage in COVID-19 is not well understood; therefore, molecular analysis of the infection process and cell signaling, *in silico*, can help in the discovery of targets for treatment of the disease.

Research objectives

To analyze liver and gastrointestinal symptoms and cell signaling pathways triggered by SARS-CoV-2 infection due to virus-liver interactions *in silico.*

Research methods

SARS-CoV-2/Liver cell interactions, and signaling pathways activated after these interactions, were analyzed by inhibition study using the molecular docking method.

Research results

mTORC1/CC-223 complex, Akt/MK-2206 complex and Furin/Nafthofluorescein complex showed significant affinity energy, indicating stability and consequent effectiveness in the process of inhibition of target molecules for COVID-19 therapy.

Research conclusions

Liver disease and signaling pathways altered by SARS-CoV-2 can be modulated by inhibitors that demonstrate significant affinity of interaction with human proteins, which could prevent the progression of the infection and its symptoms

Research perspectives

Evaluate the inhibition complexes studied using molecular dynamics and verify the possibility of structural changes of the drug to increase its efficiency and avoid possible adverse effects.

73696_Auto_Edited.docx

ORIGINALITY REPORT

4%
SIMILARITY INDEX

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet	76 words — 2%
2	www.wjgnet.com Internet	37 words — 1%
3	Saleh A Alqahtani, Jörn M Schattenberg. "Liver injury in COVID-19: The current evidence", United European Gastroenterology Journal, 2020 Crossref	26 words — 1%
4	www.tandfonline.com Internet	22 words — < 1%
5	hdl.handle.net Internet	17 words — < 1%
6	Junwen Luan, Yue Lu, Xiaolu Jin, Leiliang Zhang. "Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection", Biochemical and Biophysical Research Communications, 2020 Crossref	12 words — < 1%

EXCLUDE QUOTES ON
EXCLUDE BIBLIOGRAPHY ON
EXCLUDE MATCHES < 12 WORDS