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Análise *in silico* da suposta ausência de sinergismo terapêutico na associação da hidroxicloroquina e azitromicina na COVID-19

In silico analysis of the supposed absence of therapeutic synergism in the association of hydroxychloroquine and azithromycin in COVID-19

Análisis *in silico* de la supuesta ausencia de sinergismo terapéutico en la asociación de hidroxicloroquina y azitromicina en COVID-19

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Resumo

A associação terapêutica entre Hidroxicloroquina (HCQ) e Azitromicina (AZI) foi considerada como terapia para COVID-19, no entanto, não está claro se uma ação sinérgica ocorre. Para entender melhor sobre essa associação terapêutica esse trabalho teve como objetivo analisar a interação da HCQ e AZI com receptores humanos *in silico*. A análise foi realizada por meio de simulação de acoplamento molecular. As interações químicas entre a HCQ e a AZI com seus os prováveis receptores no organismo humano, ACE2 e CD147, foram analisadas no software AutoDock Vina e os resultados foram analisados no software PyMol. Os confórmeros HCQ-ACE2 e AZI-CD147 foram formados com energia de afinidade significativa de -7,0 Kcal/mol e -7,8 Kcal/mol, respectivamente. Embora a interação entre HCQ e ACE2 possa prevenir a invasão das células pelo vírus, essa interação pode levar a efeitos colaterais graves. Por sua vez, a interação AZI-CD147 também pode atuar na prevenção da entrada do vírus nas células. Além disso, de acordo com os dados *in silico*, a interação AZI-CD147 ocorreria de forma mais eficaz, o que leva a crer que a ação terapêutica da HCQ no COVID-19 não é tão relevante quanto à ação do AZI e não haveria sinergismo.

Palavras-chave: ACE2; Azitromicina; CD147; COVID-19; Hidroxicloroquina; Simulação de acoplamento molecular.

Abstract

The therapeutic association between Hydroxychloroquine (HCQ) and Azithromycin (AZI) has been considered as therapy for COVID-19 however, it is unclear whether a synergistic action occurs. To better understand this therapeutic association, this study aimed to analyze the interaction of HCQ and AZI with human receptors *in silico*. The analysis was performed by the molecular docking simulation. The chemical interactions of HCQ and AZI with probable receptors in the human organism, ACE2 and CD147 were analyzed by AutoDock Vina software and the results were analyzed in PyMol software. HCQ-ACE2 and AZI-CD147 conformers were formed with significant affinity energy of -7.0 Kcal/mol and -7.8 Kcal/mol, respectively. Despite the interaction between HCQ and ACE2 can prevent the invasion of the cells by virus, this interaction can lead to serious side effects. In turn, AZI-CD147 interaction can also act by preventing the entry of the virus into the cells. Moreover, according to the *in silico* data, AZI-CD147 interaction would occur more effectively, which leads to the belief that the therapeutic action of HCQ in COVID-19 is not as relevant as the action of AZI and there would be no synergism.

Keywords: ACE2; Azithromycin; CD147; COVID-19; Hydroxychloroquine; Molecular docking simulation.

Resumen

La asociación terapéutica entre hidroxicloroquina (HCQ) y azitromicina (AZI) se ha considerado como terapia para COVID-19, sin embargo, no está claro si se produce una acción sinérgica. Para entender mejor esta asociación terapéutica, este estudio tenía como objetivo analizar la interacción de HCQ y AZI con los receptores humanos *in silico*. El análisis se realizó por la simulación de acoplamiento molecular. Las interacciones químicas de HCQ y AZI con probables receptores en el organismo humano, ACE2 y CD147 fueron analizadas por el software AutoDock Vina y los resultados fueron analizados en el software PyMol. Se formaron confórmeros HCQ-ACE2 y AZI-CD147 con una energía de afinidad significativa de -7,0 Kcal/mol y -7,8 Kcal/mol, respectivamente. A pesar de que la interacción entre HCQ y ACE2 puede prevenir la invasión de las células por virus, esta interacción puede dar lugar a efectos secundarios graves. A su vez, la interacción AZI-CD147 también puede actuar para evitar que el virus entre en las células. Además, de acuerdo con los datos *in silico*, la interacción AZI-CD147 ocurriría de manera más efectiva, lo que lleva a pensar que la acción terapéutica de HCQ en COVID-19 no es tan relevante como la acción de AZI y no habría sinergismo

Palabras clave: ACE2; Azitromicina; CD147; COVID-19; Hidroxicloroquina; Simulación de acoplamiento molecular.

1. Introduction

At the end of 2019 a sudden increase in pneumonia cases in China's Wuhan province sparked concern from experts and authorities. Initially it was suspected of an epidemic caused by etiological agents already known, such as influenza and Middle East respiratory syndrome (MERS) viruses (Perlman, 2020). However, on December 31, the Chinese government notified the World Health Organization (WHO) warning of the excessive evolution of an unknown disease in Chinese territory (Perlman, 2020). Research has intensified in the scientific environment to clarify about the pathogen that has been recognized as a new virus. The new disease was named COVID-19 and the responsible agent was called SARS-CoV-2 (Perlman, 2020; Novel, 2020).

In less than two months after the first cases, more than 70,000 infected and around

1,000 deaths were confirmed in China (Novel, 2020). Containment measures were initiated, but the virus had already spread throughout the country and the first cases outside China and the Asian continent began to emerge. By February 2020 the virus had spread to 28 countries, including Europe (Novel, 2020). The transmission between countries quickly and widely caused the disease to be declared a pandemic by the responsible agencies on March 11st, 2020 (WHO, 2020). By July 12nd, the disease had spread worldwide with 12,552,765 confirmed cases and 561,617 deaths, with the United States, Brazil and the United Kingdom being the countries with the highest number of cases (Worldometers, 2020).

The symptomatology of COVID-19 is extremely variable and is not fully understood (Lima, 2020). A large portion of those who contracted the disease have mild and/or moderate symptoms. In patients with more severe symptoms, fever (87.9%), dry cough (67.7%), fatigue (38.1%), sputum (33.4%), dyspnea (18.6%), among others, are noted (Lima, 2020). Another symptom commonly found and of great clinical importance is anosmia (Lima, 2020). Pneumonia caused by SARS-CoV-2 can be divided into two phenotypes - Type H (high elastance, high lung weight, high ventilation and high shunt) and Type L (low lung weight, low elastance and low ventilation), with possible differentiation by computed tomography (CT) (Jain &, 2020). Understanding the pathological mechanisms of the disease enables the development of treatments for more individualized and effective management.

Invasion mechanism of the virus after contact with the respiratory tract is uncertain, but it is believed that SARS-CoV-2 uses the Angiotensin-converting enzyme 2 (ACE2) receptor to invade type II alveolar epithelial cells by binding to the Spike protein of the virus (Xiao &, 2020). This causes a negative regulation of ACE2 in the epithelium, leading to an increase in the concentration of angiotensin 2, which is associated with prognostic worsening in Adult Respiratory Distress Syndrome, one of the syndromes characteristic of more severe manifestations of COVID-19 (Silhol &, 2020). In addition, there is also a hypothesis that the virus uses the Differentiation Cluster 147 (CD147) or also called extracellular matrix metalloproteinase inducer or basigin receptor, an immunoglobulin superfamily protein, which also plays an important role in the infection of several other viruses, such as human immunodeficiency virus (HIV), hepatitis B virus, hepatitis C virus, among others (CUSABIO, 2020). Some experiments showed that the interaction between a structural protein spike of the virus and CD147 facilitated the entry of the virus into the host cell (Wang &, 2020).

2. Methodology

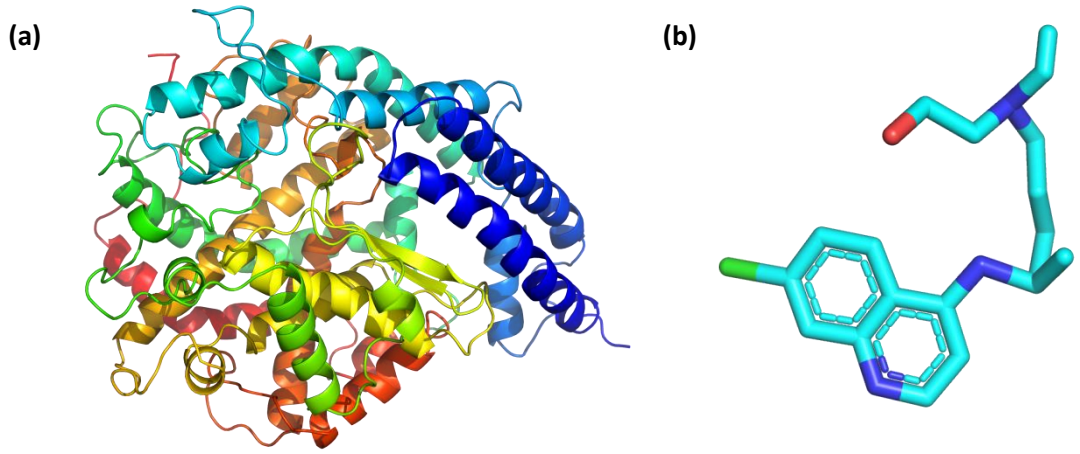
Molecular docking was performed using crystallographic structures obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/) database with x-ray diffraction crystallography method: 3BRH (CD147) and 1R4L (ACE2) at 2.80 and 3.00 Å resolution, respectively. The ligands were obtained in the Zinc (https://zinc.docking.org/) platform: Hydroxychloroquine - HCQ (ZINC1530652) and Azithromycin - AZI (ZINC85537026); theses drugs are in wide discussion in the treatment of COVID-19 disease (Gautret &, 2020).

Compounds coupled to proteins obtained in PDB were removed in the PyMol software to obtain the isolated proteins. The proteins were prepared by the AutoDock Tools software (Morris &, 2008), in which the water molecules were removed. Water molecules are derived from the crystallization process of the protein and must be removed in order to prevent them from affecting the anchoring between protein and ligand. At another stage of preparation, hydrogen atoms are added to the protein in order to add charges to all atoms since this also influences protein and ligand anchoring. In addition, the ligand torsions were detected and chosen, adapting the ligand for docking analysis. Autodock Vina software (Trott &, 2010) was used to perform the molecular docking and a grid box with dimensions (x,y,z) and specific coordinates (x,y,z) were used. The molecular docking was performed and results were analyzed in the PyMol software (DeLano, 2002).

Molecular docking aims to predict the mode of interaction of a ligand with its macromolecular target by analysis of bonds and energetic affinity between receptor and ligand; the lowest energy conformers are chosen (Raschka, 2014).

ACE2 (IR4L) and HCQ: To perform molecular docking, a grid box (x,y,z) of 90 x 80 x 90 and centralization values (x,y,z) of 39,906 x 3,091 x 22,477 were used; spacing = 1 and exhaustiveness = 15.

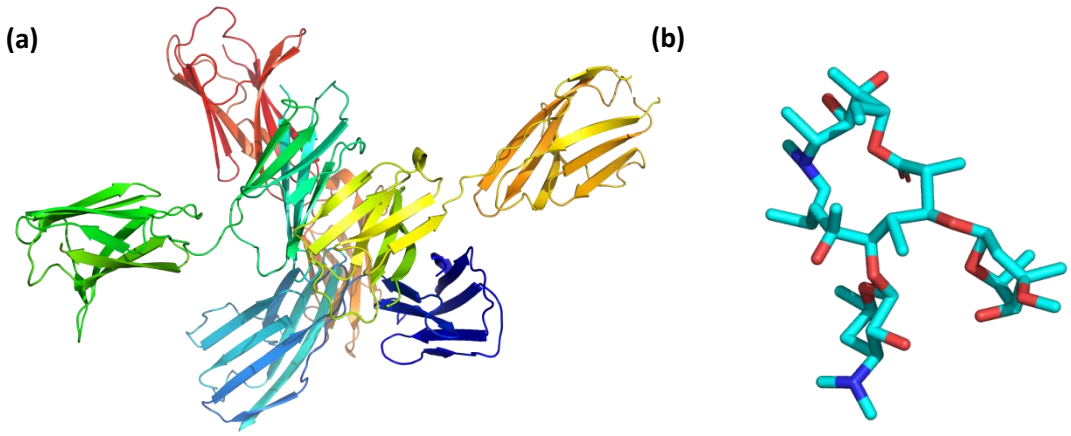
Figure 1. ACE2 (IR4L) (a) and HCQ (b) structures.



Source: Authors (2020).

CD147 (3BRH) and AZI: To perform the molecular anchorage, a grid box (x,y,z) of 126 x 104 x 104 and centralization values (x,y,z) of 11,256 x -36.44 x -14,755 were used; spacing = 1 and exhaustiveness = 15.

Figure 2. CD147 (a) and AZI (b) structures.



Source: Authors (2020)

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3. Results and Discussion

Results

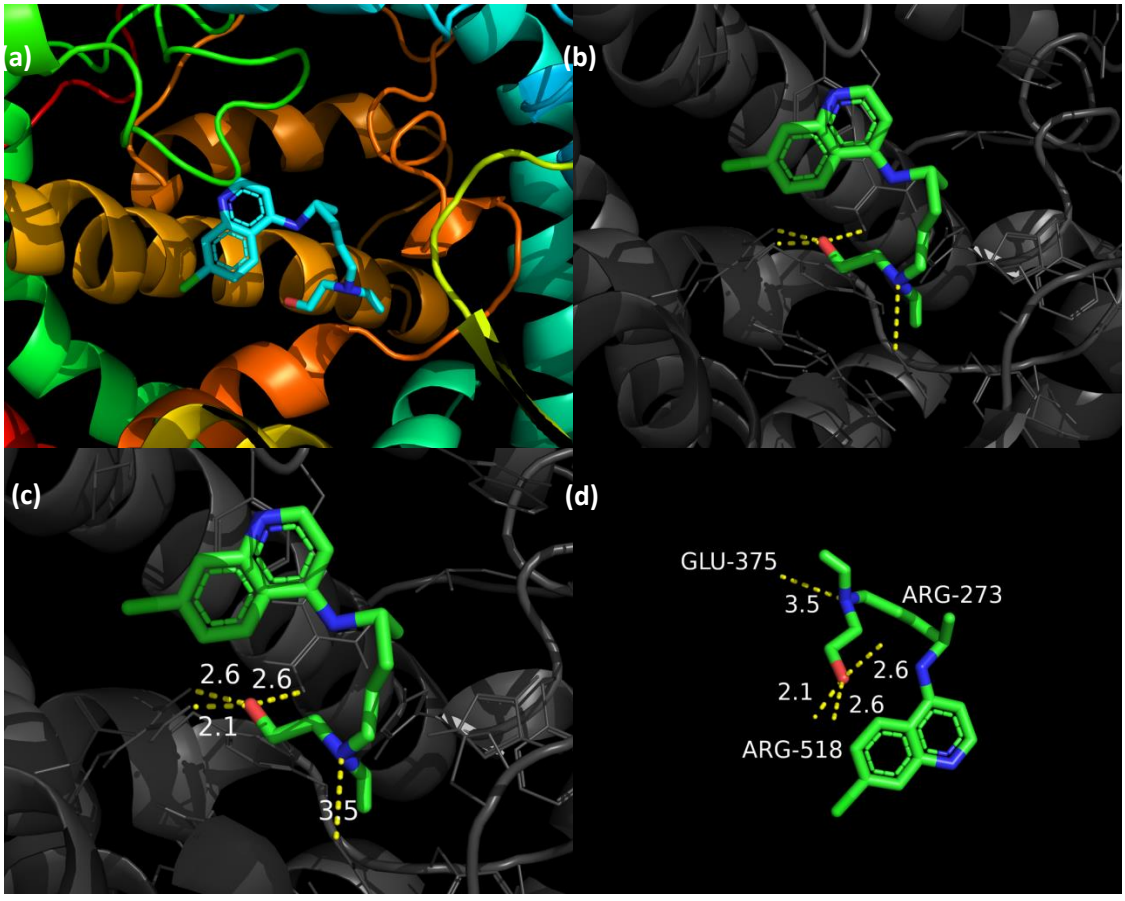
Both molecular docking simulations, HCQ-ACE2 and AZI-CD147, showed interaction between protein (receptor) and ligand. Using the PyMol software, it was possible to analyze the presence of polar bonds (Table 1).

Table 1. Polar bonds between proteins (receptors) and ligands.

ACE2 and Hydroxychloroquine			
Interaction affinity (Kcal / mol)	Ramdomization number	Involved residues	Distance
-7.0	-1374072376	GLU-375	3.5 Å
		ARG-518	2.6 Å and 2.1 Å
		ARG-273	2.6 Å
CD147 and Azithromycin			
-7.8	1108133904	ARG-106	2.3 Å, 2.6 Å and 2.8 Å
		ASP-45	3.4 Å and 3.5 Å

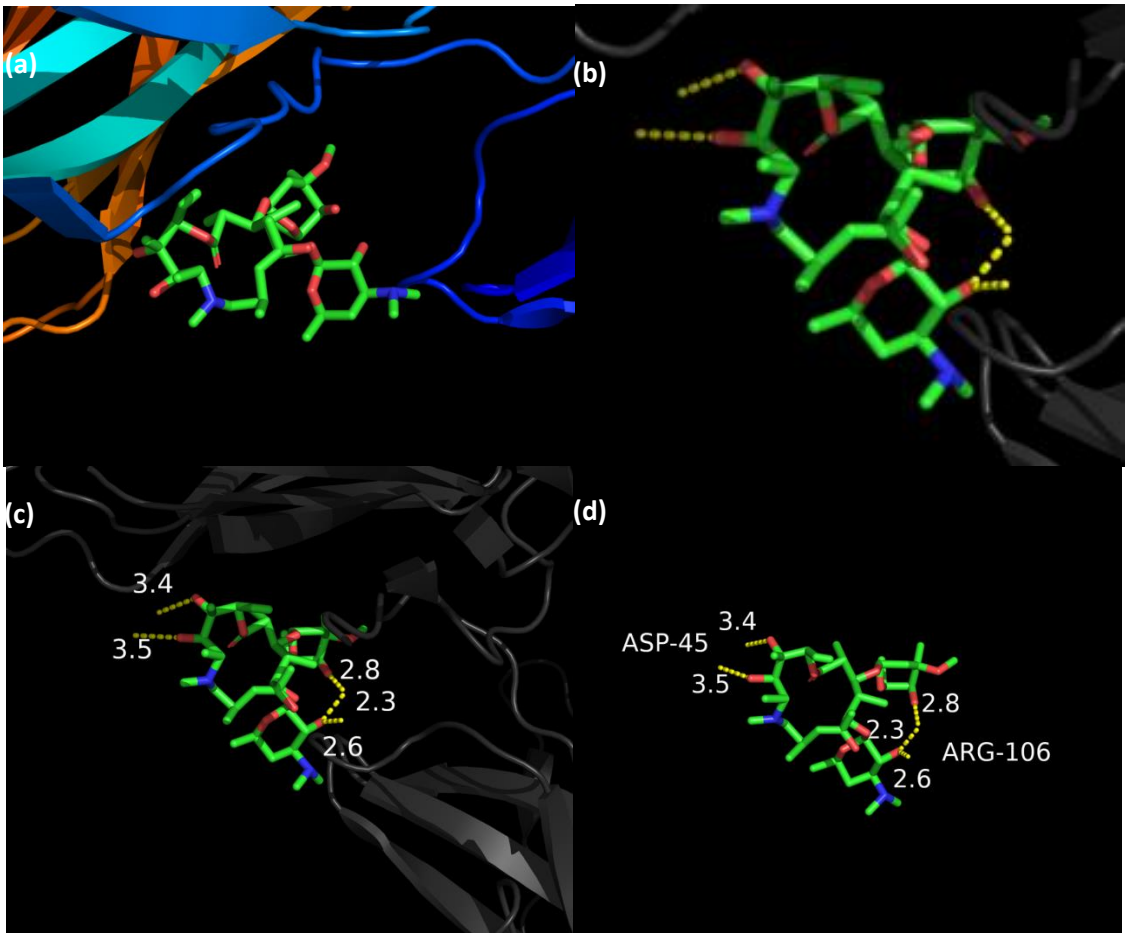
Source: Authors (2020).

Figure 3. Representation of the HCQ on its binding site on ACE2 (a), polar bonds (b), distances in Å (c) and involved residues (d).



Source: Authors (2020).

Figure 4. Representation of the AZI on its binding site on CD147 (a), polar bonds (b), distances in Å (c) and involved residues (d).



Source: Authors (2020).

Discussion

The growing concern with SARS-CoV-2, the etiological agent of COVID-19, initiated searches for drugs with potential positive effects against the disease (Gautret, et al., 2020). A study conducted in France was one of the pioneers in leveraging the discussion about the use of HCQ in the treatment of the new Coronavirus. Despite its limitations, the results obtained by Didier Raoult and his team were somewhat promising and brought hope for a possible COVID-19 treatment (Gautret, et al., 2020). However, further research has not proven the use of these drugs as beneficial (Rosenberg &, 2020).

Despite having uncertain action against the new Coronavirus (de Souza Oliveira &, 2020), Chloroquine (CQ) is a drug known and used since The World War II, when it was widely disseminated for its action in the treatment and prophylaxis of Malaria (Liu &, 2020).

After reports of cases of resistance of *Plasmodium falciparum* to CQ and because it is considered potentially toxic and harmful, this drug has gradually ceased to be used in recent years, giving space to HCQ, a derivative of CQ itself, created in 1946 and also used for malaria (Liu, et al., 2020). HCQ is less toxic and is therefore safer because it contains one more hydroxyl group than its predecessor (Liu, et al., 2020). Currently, HCQ is considered a drug with very specific recommendations and common use in patients with Lupus, Rheumatoid Arthritis and Malaria (Cooper &, 2008).

Although the therapeutic application of CQ-HCQ is old and satisfactory, its use causes important side effects, ranging from mild to severe effects that include stretching of the QTc interval and other cardiovascular problems (Sprague, 1946). A retrospective study conducted in New York with 84 people reported that in patients treated with HCQ, 30% had changes in the QT interval greater than 40 ms and 11% increase greater than 500 ms, a value considered high that confers a high risk for arrhythmia (Chorin, 2020).

Often, patients affected by diseases that require treatment with these drugs are already carriers of cardiovascular diseases, so this potential adverse effect in this population brings great concern to the medical community (Roberts &, 1999). The same happens in COVID-19, where cardiac comorbidities are risk factors for poor clinical evolution of the disease and cardiovascular adverse effects increase the chance of arrhythmias and morbidity and mortality (Zhou, 2020). Thus, a study on the mechanism of action, efficacy and adverse effects of these drugs could elucidate the best therapeutic approach.

Another frequent side effect of the use of HCQ is hyperglycemia (Brufsky, 2020). The cardiac side effect may be added to a previous comorbidity, and there is the same concern with the worsening of glycemic indices of diabetic patients, since Diabetes Mellitus (DM) was considered an isolated risk factor for COVID-19 severity (Zhou, 2020).

A study conducted through a database showed that the history of DM was seen in more ICU patients in contrast to those who did not require intensive care, demonstrating the potential risk of DM in the prognosis of the disease (Roncon &, 2020). Retinopathy is also a risk factor, leading the American Academy of Ophthalmology, in its last recommendation in 2011, to point out that the use of HCQ greater than 5mg/kg/day can cause irreversible damage to the patient's retina (Marmor &, 2011).

Due to the intense scientific discussion about this drug, potential targets of HCQ in the treatment of COVID-19 disease are being studied. Among them, ACE2 is considered to be used by both SARS-CoV and SARS-CoV-2 and, therefore, the blockade/inhibition of

glycosylation of this enzyme was associated with a potential infection prevention factor and better prognoses (South &, 2020).

ACE2 receptor is a type I transmembrane glycoprotein that converts Angiotensin 2 into Angiotensin. This enzyme is part of the renin - angiotensin - aldosterone system and has function in various parts of the body such as the heart, lungs, kidneys and brain, sites that are also commonly associated with COVID-19 symptoms (Xiao, et al., 2020).

A theory about coronavirus pathology suggests that infection, severity and prognoses of the disease depend on viral load, ACE2 concentration, and the patient's innate immunity's ability to control the progression of infection. Thus, the main target for treatment with HCQ seems to be to prevent the binding of the virus with the ACE2 receptor and consequently block the progression of the infection (Bourgonje &, 2019).

In addition to ACE2, another receptor discussed as a target for viral invasion is CD147 (Wang, et al., 2020). Similarly to ACE2, there is interaction between spike protein from virus and receptor in the host cell (Wang, et al., 2020). CD147 is a type I transmembrane protein of the highly glycosylated cell surface belonging to the immunoglobulin superfamily that acts as one of the main stimulators of matrix metalloproteinases (MMP's), plays a role in intercellular recognition as a type I integral membrane receptor (Ulrich &, 2020), and may have the level of expression increased during asthmatic and diabetic complications (Ulrich, et al., 2020). CD147 is also involved in tumor development, plasmodium invasion and viral infection, as reported by Chen & (2005), who proposes that the SARS-CoV virus (with broad genetic similarity to SARS-CoV-2) could invade the cells through CD147 (Wang, et al., 2020; Gielen &, 2010).

As a form of therapy for COVID-19, some actions of AZI with CD147 have been discussed (Crosnier &, 2011). According to Crosnier et al. (2011), CD147 is an essential receptor for the erythrocyte invasion of *Plasmodium falciparum*, and the efficacy of AZI to inhibit the invasion of this protozoan *in vitro* host cells has been reported in the literature (Wilson &, 2015). Therefore, the same blockade of CD147 by AZI could cause less invasion of the SARS-CoV virus in the host (Beigelman &, 2015).

AZI is an antibiotic of the macrolide class that has anti-inflammatory and antiviral properties with adult and pediatric dosage. The therapeutic application is old and satisfactory causing important side effects related to the gastrointestinal tract such as nausea, vomiting, diarrhea and abdominal pain (Equi &, 2002). Although it has an uncertain action to the new Coronavirus, it presents relevant benefits in inflammatory respiratory diseases such as Acute Bronchiolitis, obliterating bronchiolitis, cystic fibrosis and asthma, especially in pediatric

practice (Kuo &, 2019; Beigelman, et al., 2010). Antiviral properties were observed *in vitro* and/or *in vivo* for Ebola, Zika, Respiratory Syncytial Virus (RSV), Influenza H1N1 Virus, Enterovirus and Rhinovirus (Tran &, 2019; Kim &, 2009).

Research has elucidated the inflammatory character of COVID-19 (Beigelman, et al., 2010) disease and there are reports in the literature that inflammation tends to increase the expression of CD147 and MMP's (Kim, et al., 2009). Studies have already reported that macrolides (such as AZI) have anti-inflammatory action through an immunomodulatory response to decrease MMP's concentration and its action (Chen, et al., 2005; Chico and Chandramohan, 2011). It discusses the possibility of AZI also reducing the expression of CD147, since they have similar regulation in these processes, generating in addition to an anti-inflammatory response (Vellano &, 2020), an antiviral response; however, more studies are needed to evaluate this relationship (Chen, et al., 2005).

The association of HCQ/AZI drugs is documented in the literature for protection against malaria and other sexually transmitted diseases in pregnancy and in the child's interstitial lung disease (Breuer &, 2018; García, 2020). In this case, the patient has the benefits of anti-inflammatory activity of medications (García, 2020), which can also benefit people with COVID-19 by reducing inflammatory activity caused by the virus (Cancio &, 2020; Shukla &, 2020; Shityakov &, 2014).

The function of HCQ in Malaria is proven effective by interacting directly with *Plasmodium*, and its association with AZI causes a therapeutic synergism. Molecular docking indicates that the AZI-CD147 interaction is more stable than HCQ-ACE2 due to the higher negative affinity energy and the greater number of polar bonds. Although the ligand-receptor interaction involves many factors, binding affinity contributes to the effectiveness and speed of the interaction; therefore, the AZI-CD147 interaction was supposed to occur more effectively. Considering the divergences in the treatment of COVID-19 with HCQ, the best results obtained in association with AZI and molecular docking data, the therapeutic benefits of the association are possibly due to the anti-inflammatory action only.

4. Conclusion

The interaction between HCQ and ACE2 could prevent invasion of the host cells by SARS-CoV-2, however this interaction could contribute to the emergence of adverse effects due to ACE2 enzyme block in the renin-angiotensin system. In severe cases of COVID-19, in which an intense inflammatory condition is observed with worsening of the individual's

prognosis, AZI could prevent not only the invasion of new cells, but also preventing the continuity of activation of the inflammatory response due to interaction with CD147. According to the *in silico* analysis, monotherapy with HCQ would not present a satisfactory cost-benefit ratio for COVID-19 treatment, corroborating clinical studies on the lack of efficiency of this drug. The reported benefits on the combination therapy of HCQ and AZI are possibly not related to the interaction between the drugs or the synergism between them, but by the action of the antibiotic.

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