

Combination of Two Promising Methodologies for Possible Treatment Against COVID-19

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ABSTRACT

Objective: Combine the CRISPR-Cas13 technique with the action of the medicinal plant *Hibiscus sabdariffa* for the treatment against COVID-19.

Method: Gene and protein sequences obtained from GeneBank of the spike protein of the virus that binds the ACE2 receptor in the target cell were used. These were analyzed with the MEGA software. The chemical structures obtained from *Hibiscus sabdariffa* were also studied and compared with the structure of hydroxychloroquine and compounds that stimulate the immune response such as Acetylsalicylic acid. These obtained in PubChem.

Results: Amino acid residues that serve to bind the spike protein to the ACE2 receptor were identified and modified by changing the codons corresponding to these amino acids by stop codons in the spike protein genome. The chemical structures of *Hibiscus sabdariffa* were similar to hydroxychloroquine and Acetylsalicylic acid.

Conclusion: When the two technologies are combined, the virus could be stopped before entering the cell, due to the agonist effect of the *Hibiscus sabdariffa* chemical compounds, and in addition, the genome of the spike protein can be modified inside the target cell so that the virus runs out of infectious potential for other cells.

Introduction

SARS-CoV-2 (COVID-19) is one of the infectious agents causing the disease Severe Acute Respiratory Syndrome. SARS-CoV-2 is an RNA virus of which SARS and MERS are also part [1,2]. It is a virus that infects the person when it binds to the receptor for the angiotensin-converting enzyme 2 (ACE2) and thus penetrates its target cell. This receptor binds to the enzyme. It is involved in a series of effects such as: regulation of arterial hypertension, diabetic nephropathy, and congestive heart failure [3]. This receptor is ubiquitous in many cells (AT2 lung cells, respiratory epithelial cells, myocardial cells, cells of the esophagus and ileum, cells of the proximal tube of the kidney, and urothelial cells of the bladder) [4]. This may be one of the causes of its high spread in the body. For this purpose, the virus uses its Surface Glycoprotein S [3]. The virus could use

the ACE2 pathway to enter cells to enhance their infectivity. The immune response against the virus is characterized by a strong activation of the immune system that produces a high production of proinflammatory cytokines called "cytokine storm", which could lead to viral sepsis and inflammation with lung injury characterized by marked leukopenia, failure respiratory, shock, organ failure and the possibility of death [5-7]. In this innate immune response, we can see the mannose-binding lectin receptors (MBL) to which some viral antigens bind, which when they carry out their binding, can induce complement activation, increased phagocytosis, as well as activation of the intracellular antiviral machinery of the proteasome.

Although these responses are beneficial in counteracting viral infections, if they override and stimulate uncontrolled responses

by the immune system, they can cause septic shock and lead to widespread organ damage and death, a situation that is currently occurring in patients who die from COVID-19 infection [8]. SARS-CoV-2 is an RNA virus, therefore the best technology to attack it inside the cell is when it deposits its genetic material in the cell. The appropriate technique presented is CRISPR-Cas13, which attacks RNA and not DNA. CRISPR-13 technology has been successfully tested *in vitro* and *in vivo* and shows promise, since not only does it have the possibility of reducing the viral load in hamsters, but it may also be the only technique to avoid mutations and new strains. The possibility of using this technique should be explored either *in vivo* directly in the form of nebulization or *ex vivo* with the introduction of respiratory tract cells infected with SARS-CoV 2 [9-11]. On the other hand, medicinal plants have been used as alternative medicine for several centuries and before the revolution in drug synthesis, it was the treatment of choice in various pathologies [12]. The use of several conventional therapies for the COVID-19 coronavirus, such as ACE2 [13] receptor agonists and also hydroxychloroquine, is currently being contemplated with different results depending on the type of patient [14,15].

Although hydroxychloroquine was suspended by the WHO, in this work it was taken as a model to test *Hibiscus sabdariffa* compounds by molecular docking, as previously appreciated in its mechanism of action and considering the recommendations in Pandey, et al. [16-18]. There is a medicinal alternative derived from a plant that has been documented in the past. *Hibiscus sabdariffa*, tropical plant that receives several generic names Flor de Jamaica, Roselle, among others [19,20]. This plant has been the subject of phytochemical studies that have led to several studies on its medicinal properties [21]. It has been found that they have the ability to act on the AEC2 receptor with agonist properties [20,22]. This study intends to give a suggestion for treatment against the SARS-CoV-2 virus, combining the possibility of mutating the virus's spike protein gene, which serves as a gateway to the cell using the CRISPR-Cas13 method and the agonist response with the AE2 receptor of the medicinal plant *Hibiscus sabdariffa*.

Methods

This study was based on a systematic review of updated information on COVID-19, in the databases PubMed, Google Scholar, Scopus, GeneBank, NIH. With descriptors COVID-19, CRISPR-Cas13, Gen SARS-CoV-2, *Hibiscus sabdariffa*. The gene sequence and spike protein sequences for SARS-CoV-2 were downloaded into FASTA from GeneBank NCBI Reference Sequence: NC_045512.2. To determine the binding points of the virus to the

AE2 receptor, the sequence reported by Walls A, et al. in Cell [23] was taken as a model. With the MEGA program [24] the amino acids of the protein involved in binding to the receptor and its counterpart in the virus genome were identified. The codons for these amino acids were changed by stop codons. For the analysis of the effect of the medicinal plant *Hibiscus sabdariffa*, the structures obtained from the phytochemical study made by Castañeda R, et al. [21] and the analysis of these structures found by Barral M [25] were taken as a model. These were compared with the structure of Hydroxychloroquine [26], found in the PubChem database, as well as the compounds Mannose [27], Rutin [28], Anthocyanin Flavylium [29], Protocatechuic acid [30] and Acetylsalicylic acid [31]. Starting from the agonist effect on the AE2 receptor found in Herrera-Arellano, A. 2007 [20]. For comparison, a molecular docking was carried out between hydroxychloroquine and rutin with the ACE2 receptor and protocatechuic acid and acetyl salicylic acid with the COX 2 cyclooxygenase protein. The SwissDock online program was used [32].

Results

Based on this, here is proposed the following strategy using CRISPR technology: This is the sequence of the Virus genome downloaded to FASTA from GeneBank: NCBI Reference Sequence: NC_045512.2. corresponding to the Surface Glycoprotein S (Spike) del virus SARS-CoV-2.

```
ACTGGAAGATTGCTGATTATAATTATAAATTAC-
CAGATGATTTTACAGGCTGCGTTATAGCTTGAATTCTA-
ACAATCTTGATTCTAAGGTTGGTGGTAATTATAATTACCT-
GTATAGATTGTTTAGGAAGTCTAATCTCAAACCTTTT
GAGAGAGATATTTCAACTGAAATCTATCAGGCCGGTAGCACACCTTG-
TAATGGTGTGAAGGTTTAAATTGTTAC TTTCTTTACAATCATATG-
GTTTCCAACCCACTAATGGTGTGGTTAC
```

This is the sequence of the Virus proteins, downloaded in FASTA from GeneBank: NCBI Reference Sequence: NC_045512.2. This is the sequence of the Virus protein that corresponds to the translation of the spike protein gene

```
TGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGNYNYLRLFRK-
SNLKPFFERDISTEIYQAGSTPCNGVEGFNCY FPLQSYGFQPTNGVGY
```

According to Walls, et al. [23]: The protein sequence has amino acid residues that are those that bind to the ACE2 receptor in the target cells. These residues are seen here in the gene and spike protein. Marked in the following sequences in red [23]. Compared and modified using MEGA software.

Spike gene sequence

```
ACTGGAAGATTGCTGATTATAATTATAAATTACCAGATGATTTTACAGGCTGCGTTATAGCTTGAATTCTAAC
AATCTTGATTCTAAGGTTGGTGGTAATTATAATTACCTGTATAGATTGTTTAGGAAGTCTAATCTCAAACCTTTT
GAGAGAGATATTTCAACTGAAATCTATCAGGCCGGTAGCACACCTTGTAATGGTGTGAAGGTTTAAATTGTTAC
TTTCTTTACAATCATATGGTTTCCAACCCACTAATGGTGTGGTTAC
```

Spike protein sequence

TGKIADYNYKLPDDFTGCVIAWNS**NN**LDSKVGGN**Y**NYLYRLFRKSNLKPFRDISTEIYQAGSTPCNGVEGF**NCY**

FPL**Q**SYGF**Q**PTNGVG**Y**

The system proposed in this work is eliminate with CRISPR-Cas13 the sequence of the virus surface glycoprotein S genome (Spike) inside the cell and then be replaced by the following

UGAGGAAAGAUUGCUGAUUUAUUAAUUAAAAUUACCAGAUGAUUUUACAGGCUGCGUUUAGCUUGGAAUUCU**UGA**AAUCU**UGA**UUCU**AAG**-**GU**UGGUGGUA**UUGA**AAU**UACCUGUGA**AGA**UGA**UUU**AGGA**AGUC**UAAUCUCAAACCUUUUGAGAGAGAUUUUCAACUGAAAUCUAUCAGGC**-**CGGUAGCACACCUUGUA**UGGUGU**UGAAGGUUGAUGAUGUUGA**UUU**CCUUUAUGA**UCAU**UGGUUUCUGACCCUGAUGAUGAGUUGGUUGA**

sequence with all the binding sites changed by stop codons (It was changed the T for U to convert it into RNA from original sequence):

energies at the binding site, data not shown (Figures 1-6).

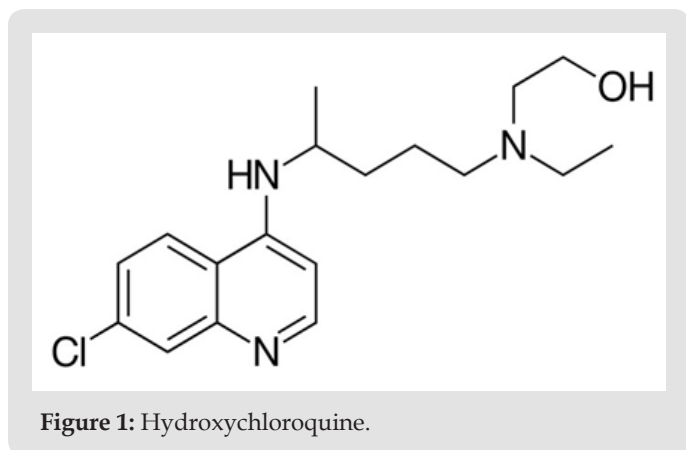


Figure 1: Hydroxychloroquine.

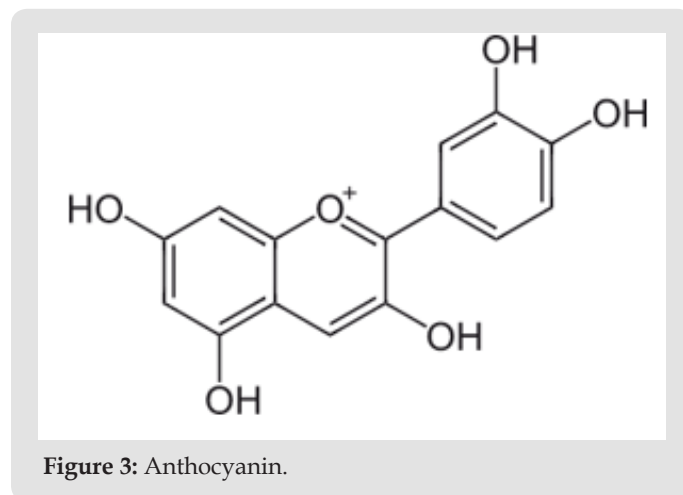


Figure 3: Anthocyanin.

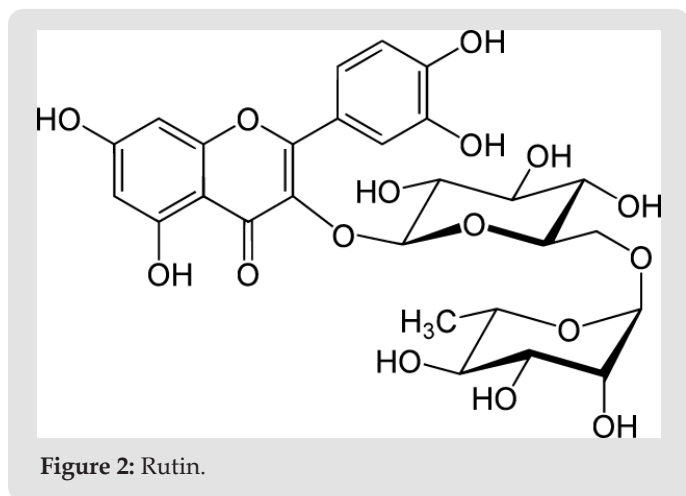


Figure 2: Rutin.

Hibiscus sabdariffa has a series of chemical compounds that have a structural similarity with hydroxychloroquine (Rutin and Anthocyanin) [25]. With high energy binding results with the ACE receptor when the two were compared. Furthermore, it has structures similar to mannose compounds that could bind to MLB receptors also in an agonist way. (Protocatechuic acid) [25]. Although the best binding energy results were presented when the molecules protocatechuic acid and acetylsalicylic acid were compared with the COX 2 receptor, protocatechuic acid was compared in Silico (Molecular Docking) with the receptor Cyclooxygenase 2 (COX2), together with acetylsalicylic acid, obtaining similar binding

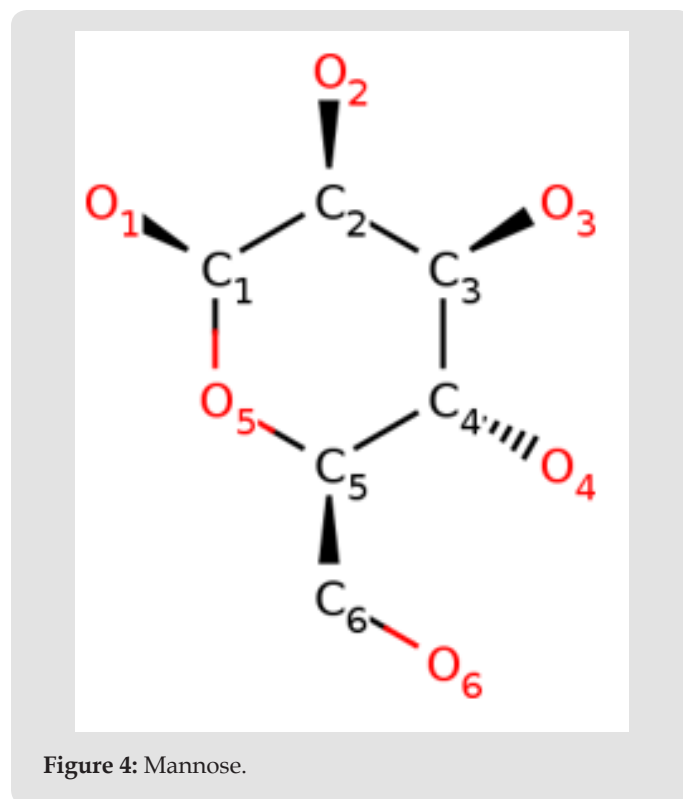


Figure 4: Mannose.

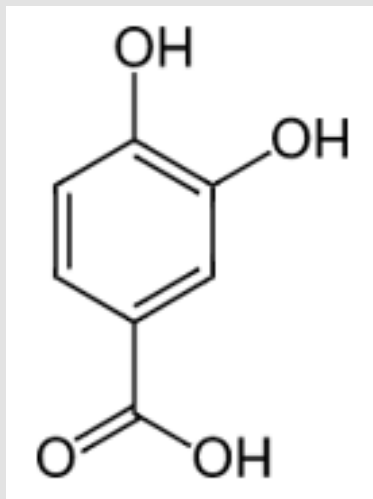


Figure 5: Protocatechuic acid.

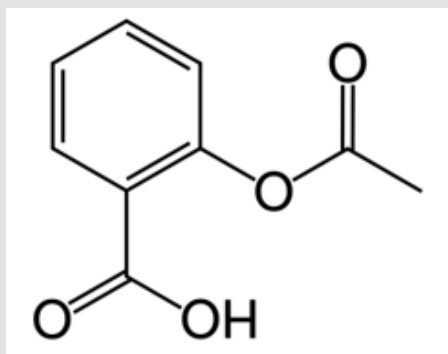


Figure 6: Acetylsalicylic acid.

Discussion

Since the entry of the virus into the cell is done by the AE2 receptor, then the genome sequence of the virus Spike protein can be changed by the mutated genome with multiple stop codons, in this way the virus could not build its protein and this would prevent it from infecting other cells. The CRISPR-Cas13 technology, unlike the other CRISPR technologies, is based on the identification of RNA sequences and not DNA [11]. This would take advantage of the fact that this coronavirus is RNA. And since its mechanism is to identify the specific sequence and replace it with another that carries Caspase13, then in this way the fragment that has the AE2 receptor binding sites could be replaced. In this way the virus will be incapacitated to infect other cells. Based on the information of *Hibiscus sabdariffa* and based on the similarity of the compounds found in the plant and the drug hydroxychloroquine [21,25], the hypothesis of the use of this medicinal plant in the control of the inflammatory response against in COVID-19 can be raised, since by affecting the ACE2 receptor, it could cause its blockage, temporarily preventing binding from the virus to the recipient and give the

immune system time to generate an adequate humoral and cellular response for its elimination and acquisition of immune memory. As an adjuvant response and by being able to block the MBL receptors, but with greater importance on its action on the COX 2 protein, blocking the main effects of this pathway, anti-inflammatories, anticoagulants and inhibition of the entire cascade of chemical mediators derived from arachidonic acid [33,34].

It could decrease the toxic shock caused by the storm of inflammatory cytokines, similar to the way that hydroxychloroquine does, which is not well known in its response in the body but its increase in immune system and decreased cytokines such as IL-1, IL-6, TNF (inflammatory triad) and increased antiviral INF-g.

Conclusion

A combination of two technologies: phytochemistry and genetic modification by the CRISPR-Cas13 technique could become a possible therapy for COVID-19 if the effects of both technologies can be combined. The medicinal plant *Hibiscus sabdariffa* has an agonist effect by attacking the receptor that uses the virus, acting as a block against the entry of the virus into the cell. To attack the virus that is inside the cell, the genome of the spike protein can be modified with CRISPR-Cas13 so that it does not occur and eliminate the contact points with the AE2 receptor and in this way the virus will be prevented can infect other target cells. Protocatechuic acid is presented as a therapeutic option in the complication of Kawasaki syndrome inherent to COVID-19 infection [35]. (Unpublished preliminary clinical data).

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