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Chloroquine and hydroxychloroquine for combating COVID-19: Investigating efficacy and hypothesizing new formulations using Bio/chemoinformatics tools

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ABSTRACT
Chloroquine (CQ) and hydroxychloroquine (HCQ) are undergoing several clinical trials for evaluating their efficacy and safety as antiviral drugs. Yet, there is still a great debate about their efficacy in combating COVID-19. This study aimed to evaluate the feasibility of intranasal and/or pulmonary administration of CQ/HCQ for COVID-19 using Bio/chemoinformatics tools. We, hereby, hypothesize the success of the intranasal and the pulmonary routes through a gelatin matrix to overcome several challenges related to CQ and HCQ pharmacodynamics and pharmacokinetics properties and to increase their local concentrations at the sites of initial viral entry while minimizing the potential side effects. Molecular docking on the gelatin-simulated matrix demonstrated high loading values and a sustained release profile. Moreover, the docking on mucin as well as various receptors including Angiotensin-converting enzyme 2 (ACE-2), heparin sulphate proteoglycan and Phosphatidylinositol binding clathrin assembly protein (PICALM), which are expressed in the lung and intranasal tissues and represent initial sites of attachment of the viral particles to the surface of respiratory cells, has shown good binding of CQ and HCQ to these receptors. The presented data provide an insight into the use of a novel drug formulation that needs to be tested in adequately powered randomized controlled clinical trials; aiming for a sustained prophylaxis effect and/or a treatment strategy against this pandemic viral infection.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection or COVID-19 has now been declared by the WHO as a new pandemic disease and hence contingency measures are urgently needed to face this immediate global challenge. Given the novelty of the infection and the lack of preventive vaccines or proven treatments, drug repurposing is being currently employed to combat this worldwide outbreak. This is an emerging strategy where existing medications that had already been tested safe in humans, are redeployed to combat difficult-to-treat diseases.

Chloroquine (CQ) and its derivative, Hydroxychloroquine (HCQ), have been used as prophylactic measures in malaria-endemic regions and also for the treatment of autoimmune diseases world-wide. CQ has shown therapeutic activity against viruses [40] including the human corona virus OC43 in animal models [29] and SARS-CoV in cell cultures [45]. Both CQ and HCQ were recently shown to have similar anti-SARS-CoV-2 effects in cell culture [32,59] and so may have prophylactic and/or therapeutic effects against COVID-19 [8]. Recently, Wang et al. demonstrated that at a low micromolar concentration, CQ was able to potently block viral infection at both entry and at post-entry stages of the SARS-CoV-2 in vitro. The effective concentration was that achievable in patients receiving 500 mg daily [49].

Recent evidence suggested that SARS-CoV entry (being a nanoparticle) [63] into host cells involves receptor-mediated endocytosis [64] despite that it was initially thought to directly fuse with the plasma membrane [48]. Heparan sulphate proteoglycans (HSPGs) were shown to provide the preliminary docking sites for the spike protein on the viral cell surface [46] and play an important role in SARS-CoV invasion at the early attachment phase [30]. The human Coronavirus NL63

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CQ anti-SARS-CoV activity was demonstrated in the cell cultures even when administered after a viral infection [45]. This includes inhibition of pH-dependent viral fusion/repliocation by increasing the pH of endosomes and lysosomes [60]; prevention of glycosylation of the viral glycoprotein envelope and the host receptor protein [13]. Besides, CQ caused the inhibition of the virion assembly [9,40].

In addition to their direct antiviral actions, CQ and its derivatives were proven to exhibit host effects by attenuating the expression of pro-inflammatory factors and receptors [10] that can induce acute respiratory distress syndrome and are involved in the cytokine storm associated with the disease severity of COVID-19 [25]. Moreover, CQ and HCQ have demonstrated not only anti-viral and anti-inflammatory effects but also anti-thrombotic effects [39].

Nevertheless, CQ was proven to act as a zinc ionophore [57], where this element acts against viral replication by interfering with the viral RNA-dependent RNA-polymerase [28].

The efficacy and safety of CQ/HCQ in the clinical context of treatment and prophylaxis of COVID-19 were addressed in several clinical trials that showed inconsistent results. A clinical study with more than 100 patients demonstrated superiority of CQ phosphate to controls in inhibiting the exacerbation of pneumonia, and shortening the disease course without severe adverse events [13]. A non-randomized open-label trial in 20 patients showed that HCQ induced viral clearance in nasopharyngeal samples after 6 days of treatment, either alone or in combination with azithromycin [14]. On the other hand, an observational study involving 1446 hospitalized patients with COVID-19 showed that HCQ administration was not associated with either a greatly lowered or an increased risk of the composite end point of intubation or death [15]. A recently published systematic review concluded that COVID-19 patients should be treated with CQ/HCQ only if appropriately monitored and if the treatment lies within the context of high quality randomized controlled trials [11].

Understanding the mechanisms by which CQ and HCQ affect SARS-CoV-2 would be critical for optimizing and developing preventative and therapeutic strategies [24]. We sought to evaluate CQ and HCQ as potential anti-SARS-CoV-2 using Bio/chemoinformatics tools aiming to provide a help in the debate. The differences between the two investigated molecules (if any) could be attributed to the differences in the main structural, topological and electronic descriptors of the drugs [20,35]. Both drugs were evaluated at the therapeutic level pertaining to suppression of endocytosis in host cells through studying the interaction with HSPGs, ACE-2, and PICALM. They were also evaluated at the formulation level by comparing the loading in gelatin microsphere matrices as a basis for the formation of particulate systems (micro and nano); and at the biopharmaceutical level through studying the interaction with mucin.

CQ and its derivatives are reported to possess high volume of distribution after oral administration reaching a level of 100 l per one kg of the patient weight indicating a high distribution in the tissues compartment; and therefore have to be administered in relatively high oral dosages; a fact that leads to the associated side effects [19]. These effects may range from ocular toxicity [41] to myocardial infarction [5]. This may in turns limit its clinical use and hamper successful treatment of COVID-19 patients.

To this end, the aim of the current study is to evaluate the feasibility of a CQ/HCQ formulation that would be administered through an alternative route other than the oral delivery such as the intranasal and/or pulmonary routes. Uniting re-purposing of drugs with re-formulation aids in increasing the local concentrations of the drugs at the sites of initial SARS-CoV-2 entry and leads to a sustained prophylaxis effect and/or as a treatment strategy, while potentially reducing the adverse events.

2. Methodology

2.1. Construction of the virtual carrier using molecular dynamics simulations

GROMACS (17) v4.6.5 software package was used to carry-out all-atom molecular dynamics simulations. The atom typing and assignment of parameters and charges of the gelatin matrix [35] were carried-out online (https://cgenff.paramchem.org/) according to CHARMM general force field (CgenFF). To prepare the gelatin system, 48 peptide molecules were constructed, comprising 18 amino acids in each molecule. The primary sequence of the peptides was AGPRGQ (Hyp) GPAPDGDQ (Hyp)GP. The matrix was then subjected to a molecular dynamics run, with full periodic boundary conditions, a time step of 2 fs, and a cut-off distance for van der Waals’s and electrostatic interactions of 1 nm. PME was used to calculate electrostatic interactions and LINCS algorithm was used to constrain all bonds. The systems were equilibrated for 7 ns at 298K using a v-rescale thermostat at a pressure of 1 bar using a Berendsen barostat.

2.2. Construction of the Heparan Sulphate proteoglycan (HSPG)

The chemical structure of HSPG was obtained from PubChem®, drawn using the ChemDraw® Ultra package version 10 where two molecules (simulating the HSPG present in cell membranes) were utilized for docking after energy minimization using MMFF94x forcefield found in MOE® version 2014.0901 (Chemical Computing Group Inc., Montreal, Canada).

2.3. Obtaining the target peptides and proteins virtual matrices

The crystal structure of the relevant delivery and the related receptor targets were obtained from the protein data bank (http://www.rcsb.org). The codes 2ACM corresponded to Mucin. The codes 6m17, 3zyk corresponded to the ACE-2 and PICALM, respectively. The polar hydrogens were added to the obtained pdb files using MOE® version 2014.0901 (Chemical Computing Group Inc., Montreal, Canada).

2.4. Preparing the drugs chemical structures for docking

The isomeric SMILES corresponding to the chemical structures of the studied antimalarial drugs; CQ and HCQ were obtained using Pub-Chem®. The corresponding 3D chemical structures were generated using the builder function of MOE® version 2014.0901 (Chemical Computing Group Inc., Montreal, Canada). Further, energy minimization was carried out for all the investigated molecules using MMFF94x forcefield of the same software [21].

2.5. Docking of the investigated drugs on the investigated carrier

The docking analysis was employed using MOE version 2014.0901 (Chemical Computing Group Inc., Montreal, Canada). The pdb file of the protein nanoparticles matrix was imported to MOE where the identification of the binding site was performed using MOE’s “Site finder” tool [23]. The docking experiment was conducted using the “triangle matcher” as a placement method.

The used software creates dummy atoms around the docking target.
3. Results and discussion

The global impact of COVID-19 infection is profound as it represents a public health threat that is entering a phase beyond containment. De novo drug development is a lengthy and a costly process and hence impractical to face the immediate global demand. The antimalarial drugs, CQ and HCQ, have shown to block COVID-19 infection in cell culture studies [50,59]. CQ, specifically, was recently found to be highly effective in reducing the viral replication that can be easily achievable with standard dosing due to their favourable penetration in tissues such as the lung bib33 [7,33].

Deepening in the several suggested mechanisms by which these drugs could affect SARS-CoV-2 would provide a more profound insight into the rational formulation that is needed for the treatment of this infection. In this study, Bio/Chemoinformatics tools are deployed to study the docking of CQ and HCQ into a gelatin matrix as the basis for the formation of particulate systems (micro/nano). Table 1 shows the obtained binding energies after docking of the two investigated drugs HCQ and CQ on the related macromolecules.

Both molecules showed good interactions with the investigated carrier and receptors. After performing an un-paired t-test using GraphPad Prism® v.5.0, the slightly significant (P < 0.05) lower score of the binding energy (more negative values of ΔG) indicated better interaction of HCQ on the gelatin matrix. This could be attributed to its more hydrophilic nature demonstrated by its higher total polar surface area and more H-bond donors and acceptors that can interact with the gelatin carboxylic and amino groups [22,38] compared to the CQ counterparts. This higher interaction may result in a higher loading potential and more sustained release effect of HCQ, specifically, from the gelatin matrix. Gelatin has been rationally selected as the nanoparticulate matrix material for loading the investigated drugs due to its high biocompatibility, suitability for nasal and pulmonary delivery, mucoadhesive properties and its efficiency on loading several hydrophilic and hydrophobic drugs [1,22,42]. The successful construction of the gelatin matrix using the adopted molecular dynamics method was obtained after following the same protocols of the authors previous studies [23,35].

On the delivery level, the docking results of the two drugs on mucin as an indicator of the ability of the drug for better mucoadhesion was also in favor of HCQ. Mucin is an abundant micromolecule that is present in the airways [47]. Furthermore, higher affinity of HCQ to HSPGs, ACE-2 and PICALM was observed as revealed by the lower binding energy scores. This may be attributed to the higher polarity and hydrophilicity nature of HCQ as demonstrated by its physico-chemical descriptors; higher total polar surface area, lower LogP value and higher numbers of H-bond donors and acceptors (Table 2). These are crucial properties that are warranted for the compatibility with hydrophilic amino acids that are present in mucin, ACE-2 and PICALM such as: Aspartic acid, Glutamic acid, arginine and phenylalanine. Moreover, other HCQ structural and topological descriptors such as: less globularity, higher molecular flexibility and lower molecular weight contribute to the overall better affinity and interaction whether with the aforementioned enzymes or with HSPG (Table 2). Nevertheless, the obtained binding energies (Table 1) for HCQ specifically indicate significant interactions with the investigated macromolecules and high affinity (Fig. 1).

HSPGs act as primary binding sites, promoting viral docking and facilitating subsequent interaction with the specific receptors and also function as storage sites on non-permissive cells mediating “in trans” infection by presenting the viruses to their target cells [46]. Furthermore, Heparan sulphate is present in the lung tissues and is necessary for pulmonary haemostasis [17]. ACE-2 has been shown to be a co-receptor for SARS-CoV-2 viral entry with increasing evidence that it has a protected role in the pathogenesis of COVID-19 [61]. ACE-2 is expressed in many body tissues, including the lungs [27,56,62]. CQ can interfere with ACE-2 receptor glycosylation thus prevents SARS-CoV-2 attachment to the target cells [33,49]. PICALM shows medium distribution in nasopharynx, bronchial and lung tissues (https://www.proteinatlas.org/ENSG00000073921-PICALM/tissue). PICALM is one of the three most abundant proteins in clathrin-coated pits. The depletion of this receptor has previously been shown to inhibit clathrin-mediated endocytosis; an important mechanism for the SARS-viruses internalization [24,37]. This is considered the main mechanism of nanoparticles internalization as well [2,12]. Thoughtfully, exploiting nanoparticulate formulations can impose competitive inhibition with the virus entry. It was previously shown that binding of CQ and HCQ to PICALM may induce the down-regulation of this protein [51].

The safety of CQ/HCQ is well established in malaria or autoimmune disease. However, some serious side effects (cardiotoxicity, retinopathy and neuromyopathy) may occur with long-term usage of high doses or in overdose [3,18,19]. A clinical study of CQ phosphate in the treatment of COVID-19 associated pneumonia did not show severe adverse reactions in the patients [13]. On the other hand, in a cohort study of 90 hospitalized COVID-19 patients, the use of HCQ with or without azithromycin for treatment of pneumonia associated with COVID-19 increased the risk of corrected QT (QTC) prolongation, and concurrent treatment with azithromycin was associated with greater changes in QTc [34]. COVID-19 patients could be more vulnerable to these side effects because of their co-morbidities (such as cardiovascular disease, diabetes and obesity), advanced age, and subsequent co-medication [16].

4. Hypothesized prophylaxis protocol

Both CQ and HCQ have shown to have good binding scores to the aforementioned receptors as shown in Table 1. Furthermore, in an
interesting study conducted by Bernard et al. [4], the intranasal CQ was proven effective in reducing the SARS-virus titres in BalB/c mice treated with 30 mg/kg dose by about a one-half logarithmic magnitude. In a more recent study, SARS-CoV-2 entry factors were found to be highly expressed in the nasal epithelial cells [43]. This finding encourages and warrants the usage of the intranasal route of administration of the suggested virus combating drugs. Hence, we propose testing the intranasal and the pulmonary routes in ongoing clinical trials as alternative routes to the oral delivery. The advantages of administering the drugs directly to the sites of viral entry include higher drug concentration in the affected tissues (the lungs and intranasal tissues) while avoiding large systemic concentrations [6], and minimizing the potential side effects as well. This is of great benefit for any prophylactic treatment that requires prolonged administration of a drug, particularly as in the case of CQ or HQ.

According to the FDA, the therapeutics that are administered through alternative routes to the intravenous and the oral routes, should be normalized to the area of application [44]. The human intranasal and pulmonary areas are taken as 5 times those of the Balb/c mice. Moreover, it is now well acquainted that the intranasal and pulmonary doses are more effective than their oral counterparts and hence down-regulation of the doses is usually attained [55]. Therefore we suggest a daily dose of 50–100 mg CQ or HCQ [58] administered intranasally or through inhalation in a combination of gelatin micro/nanospheres matrices together with a zinc oral supplementation therapy [28]. The microspheres would impart better contact and localization of the drug associated with a sustained release effect. Complementary to the microspheres action, the nanospheres would suggest another mechanism for combating the virus through competitive inhibition with the virus via the PICALM mechanism and would also impart better penetration into the lung cells (in case of treatment purposes).

5. Conclusion and future perspective

We hereby suggest evaluating the efficacy and safety of the use of CQ or HQ through the intranasal and the pulmonary routes in adequately designed and powerful clinical trials aiming for the prophylaxis and/or the treatment of COVID-19. We proposed a novel idea of formulating these related re-purposed drugs in a combined gelatin micro/nano particulate matrices systems that would be administered through the intranasal and the pulmonary routes. This will lead to the prevention and the competitive inhibition of the cellular viral invasion in one hand and to the increase in the drugs penetration, the enhancement and the sustainment of their antiviral activity while reducing their dosing and hence the unfavourable side effects on the other hand. It is worth noting, that this virtual approach could be beneficial in assessing any other re-purposed drugs or new molecules, not only on the biological but also on the formulation level.
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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