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Detailed comparison between the safety profiles of chloroquine and hydroxychloroquine

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Abstract: Introducing an innovative drug to the market requires not only large amounts of money, but also time. Therefore, the process of repositioning, i.e., finding new indications for drugs already in use, is becoming more and more frequent. In 2020, chloroquine (CQ) and hydroxychloroquine (HCQ) were approved for the treatment of severe COVID-19 infection. However, studies have shown, that the adverse effects of these drugs are too serious and the approval was revoked. The most serious and most frequently reported adverse effects were cardiotoxicity and neurotoxicity. For this reason, the study investigates the possible mechanism of cardiotoxicity and neurotoxicity of CQ and HCQ using in silico computational methods. The results of this study were found to be consistent with the literature.

Keywords: in silico methods; quinoline derivatives; neurotoxicity; cardiotoxicity

1. Introduction

Over the last decades, we have seen a decrease in the number of new medicines introduced to the market. This is primarily due to the high costs of research and the length of the various stages of new substance testing [1]. Only 48 drugs have been approved for use by the Food and Drug Administration (FDA) in 2019 [2] and another 53 in 2020[3] Moreover, 2018 is considered to be one of the record-breaking years, where this number reached a value of only 62 [2] Despite the constant development of medicine, the number of new medicines and the indications for which they can be applied is still insufficient to provide effective treatment for any condition. In order to improve the effectiveness and quality of pharmacotherapy, researchers are increasingly emphasizing so-called repurposing (or repositioning, reprofiling or re-tasking), i.e., searching for new indications for drugs already in use [4]. The method requires activities within two areas of drug development: in silico studies, based on drug-drug and drug-target interactions, and experimental including in vitro and in vivo assesses. Connecting these two areas provides an opportunity to reduce the costs associated with introducing a new treatment method and significantly accelerate the process. Repositioning is developing for a multitude of reasons. Firstly, the drug, which has already been authorized for marketing, has undergone all safety tests, has passed all phases of clinical trials, and therefore the risk of causing adverse effects is reduced significantly. Frequently, the most suitable form of administration is already known, which significantly speeds up the whole process of introduction the drug medication. Certainly, the costs of phase I and II clinical trials are reduced. Additionally, this method offers the possibility of discovering new therapeutic targets for investigational drugs. All the above arguments prove that repurposing brings many benefits for both patients and pharmaceutical companies [5]

The well known example of such a drug is Sildenafil, which was originally tested for the treatment of angina pectoris, but turned out to have more measurable effects when used as an erectile dysfunction drug. Another example is minoxidil, which was originally designed to treat ulcers but has been applied as a hair growth drug [6]. The utilization of this method also found application in March 2020, when the COVID-19 pandemic caused by the SARS-CoV- 2 virus was announced by the World Health Organization (WHO). Among the drugs used *off-label* were CQ (N'-(7-chloroquinolin-4-yl)-N,N-diethylpentane-1,4-diamine, trade name: Aralen; Arechin) and HCQ (trade name: Plaquenil). Both CQ and HCQ were approved by the FDA for the use of the above mentioned virus in emergency situations[7]. These pharmacotherapeutics have long been of considerable interest to the scientific community due to the phenomenon of hormesis observed during therapy with these drugs [8] A multitude of scientific studies have confirmed that both these drugs are highly effective in controlling the infection caused by SARS-CoV-2, reducing the development of pneumonia and thus shortening the duration and intensity of the disease [7] However, on June 15th 2020, the FDA decided to withdraw these drugs from emergency use authorization (EUA) for Covid-19 therapy due to the risk of cardiotoxicity [3] This news caused a stir among patients and scientists alike. The main source of controversy was the FDA's ambiguous and shifting stance on the use of CQ and HCQ. In addition, the mechanism of the observed prolongation of the QT interval of the action potential following administration of CQ and HCQ has not been clearly defined. Nevertheless, some researchers suggest that one of the possible causes of this phenomenon may be the influence of these drugs on the activity of cardiac ion channels [9,10]

1.1. Ion channels

Ion channels are protein molecules dispersed in the cell membrane, whose role is to transport ions through a lipid bilayer. Special attention of scientists is focused on *Voltage-Gated Ion Channels* (VGIC) which have been among the most common molecular drugs targets [11] Due to the fact that the risk of cardiotoxicity has been a frequent reason for withdrawal of drugs from the market. In 2005 studies were undertaken to explain the causes of cardiotoxicity. This extensive research as the source of the two most commonly reported disorders, i.e., torsade de points and QT elongation of action potentials, has shown blocking potassium heart channel, which is encodes by the human ether related gene (hERG gene). This allowed for the inclusion in the basic safety assessment process of new drugs of hERG blocking studies which consequently significantly limited drug withdrawal for this reason [12] Nevertheless, the new guidelines on drug cardiotoxicity studies proved that not only hERG but other VGIC channels are the site of drug action, and disturbances of VGIC-drug interaction may be a probable cause of cardiotoxicity development. The most important ion channels proposed in the comprehensive in vitro proarrhythmia test (CiPA) include: $K_v11.1$ (hERG), $Na_v1.5$-late and $Ca_v1.2$, $K_v4.3$, K_vLQT1/mink and $K_{ir}2.1$. Their influence on the repolarization and depolarization of the heart action potential (AP) has been confirmed and proven in numerous tests covering the physiological state and pathological state of the human organism [13] However, the first three channels are considered the most crucial in assessing the risk of cardiotoxicity.

2. Methodology

It should be emphasized that the safety requirements for medicines have become more stringent over the years. At the same time, new methods of computational chemistry, based on the structure of drugs and their similarity to already known active molecules, allow early identification of potential adverse interactions.

2.1. In silico methods

In this work the in silico methods were applied the answer the question whether CQ and HCQ can interact with voltage-gated ion channels in the heart. ProToxII [14] and AdmetSAR software [15,16] was used to calculate the probability of induction of toxicity in selected areas of activity. The mechanisms of action of the studied drugs in the

cardiovascular and nervous systems are underlined. In order to compare the cardio and neurotoxicity of the investigated drugs, their full safety profiles were tested.

Using the ProToxII software, one can determine the properties of already known molecule structure by using its name or drawing the structure. The calculations included in this study present the results of the oral toxicity predictions. A full safety assessment report is also prepared based on the likelihood of toxicity or non-toxicity in a particular activity model

According to statistical studies one of the most common reason for suspension or withdrawal of drugs from the market is the risk of cardiotoxicity and neurotoxicity [17] Unfortunately, one of the drawbacks of the ProToxII program is lack of the information of potential neuro- and cardiotoxic effects of calculated compounds. Therefore, in the analyzes present the AdmetSAR program was used to test the safe application of test compounds on the heart and neuronal system. It is worth emphasis that the computational algorithms of both programs are very similar [18] In both algorithms, the results of toxicity probability yield from a comparison of the test molecule with data collected in available compound databases and literature containing 717 toxic molecules (IC_{50} < 30 μMol) and 216 non-toxic particles [19] The specific toxicity model is created by using an algorithm called AtomPairs coded with the appropriate vector notation. It allows to assess a drug administration safety profile, based on the chemical structure of the compounds. Full assessment of the pharmacological safety profile of the studied drugs is possible combining results of ProTOX II and AdmetSAR. The resulting data are shown in Figure 1 as juxtaposition of the toxicity profiles of studied compounds with the average values of the probability of inactivity for substances in the considered toxicity class of the model/molecule.

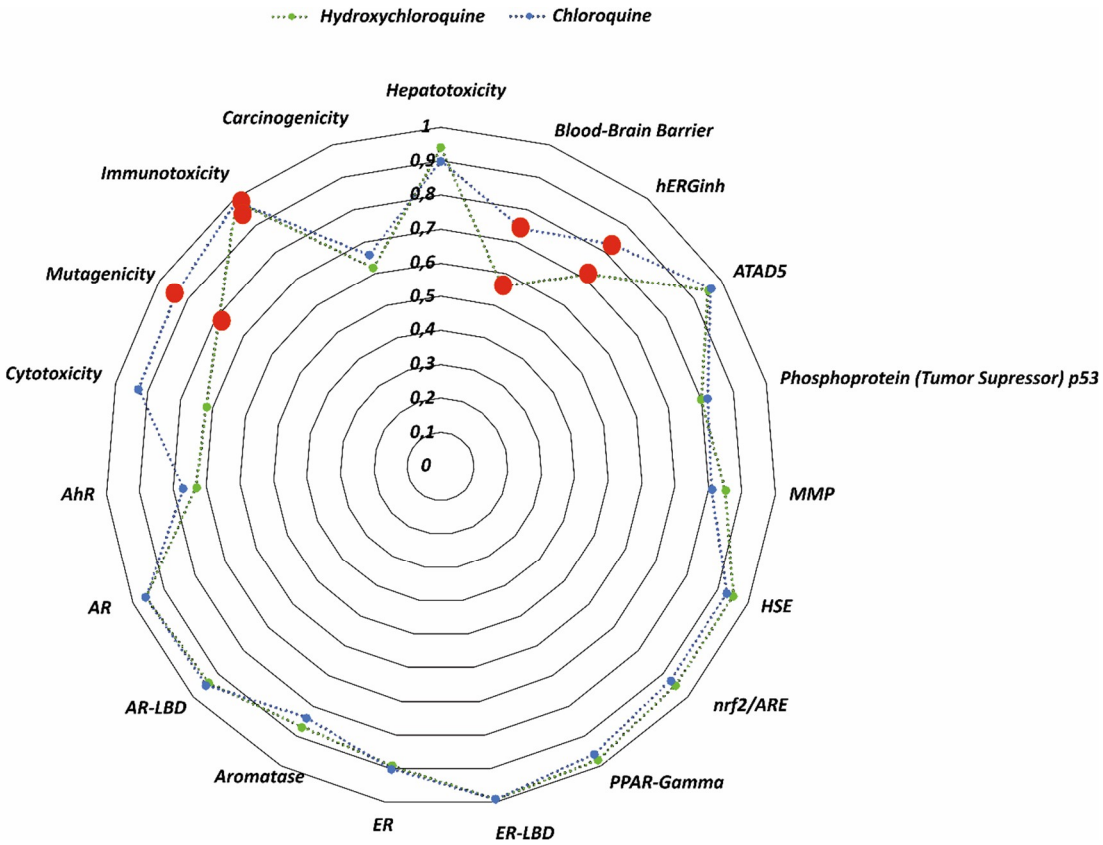

Figure 1. Comparison of the safety assessment of CQ and HCQ. The markers indicate a higher probability of toxicity of the tested molecules in comparison with reference substances. The symbols indicate: hERGinh - the human Ether-à-go-go- Related Gene, ATAD5 - ATPase family AAA domain-containing protein 5, MMP - Mitochondrial Membrane Potential, HSE - Heat shock factor response element, nrf2/ARE - Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element, PPAR – Gamma - Peroxisome Proliferator Activated Receptor Gamma, ER – LBD - Estrogen Receptor Ligand Binding Domain, ER - Estrogen Receptor Alpha, AR – LBD - Androgen Receptor Ligand Binding Domain, AR - Androgen Receptor, AhR - Aryl hydrocarbon Receptor.

Both drugs have been reported to be active at the same toxicity levels/types. The pharmaceuticals exhibit immunotoxic properties with a 99% probability. The study indicates that the risk of mutagenicity for CQ is higher (94%) compared to HCQ (79%). Probability results obtained using AdmetSAR program provided information on the inhibition of the hERG gene. The probability of QT prolongation is: 83% for CQ and 71% for HCQ. Moreover, the substances are highly likely (74% for CQ and 68% for HCQ) to pass through the blood-brain barrier (BBB). Attention should also be paid to the risk of carcinogenicity - calculations show that both drugs should not participate in tumorigenesis. However, the probability of no toxicity in this area is for CQ only 66%, while for HCQ 62%.

3. Discussion

The in silico studies conducted within this project provide a preliminary view of the potentially toxic areas of action of CQ and HCQ. The data are particularly important in changing the pharmacotherapeutic position of drugs, i.e., discovering new or extended uses of clinically available drugs. Of course, one should remember that this type of research cannot replace animal testing, but it can significantly limit their number. By modulating cell excitability and altering action potentials, quinoline derivatives have been reported to affect excitable cells (both neuronal and cardiac) In our discussions, we decided to confirm the result of the neuro- and cardiotoxicity of the tested derivatives.

3.1. Neurotoxicity

Tests showed that quinoline derivatives easily pass through the blood-brain barrier, which was confirmed by literature data [20] This ability allows the tested drugs to exert effects in the nervous system. Studies have demonstrated that CQ and HCQ affect the secretion and effect of neurotransmitters such as serotonin[21] and adenosine[22]. These compounds can also affect opioid receptors, which is related to the possibility of drug accumulation in the central nervous system (CNS). Dysfunction of these neurotransmitters may lead to abnormal communication between cells.Additionally, inhibition of the neurotransmitter γ amino butyric acid (GABA) may lead to brain dysfunction (encephalopathy) [23]

The action of CQ in the nervous system is also manifested by the effects on lowering and raising the convulsive threshold. According to the collected data, in low doses ((1.0–5.0 mg/kg) the drug increases the seizure threshold, but in high doses ((10.0–50.0 mg/kg) it has a pro- convulsant effect. According to data, this effect may be related to the influence of CQ on the opioid system [24,25]

Adverse effects after CQ intoxication are manifested by extrapyramidal nervous system symptoms. These include ataxia, dysphonia, involuntary tilt of the eyes up (oculogyric dystonia). Most of the symptoms resulting from CQ-inhibition are reversible, but chronic neurological disorders such as temporal lobe epilepsy and dysautonomia may develop. Most neurological symptoms disappear when QT is discontinued, but studies in animal models have reported that CQ can cause permanent brain and brainstem damage [23]

Free passage through the blood-brain barrier also made it possible to use CQ as a neuroprotective agent. Very low concentrations of CQ can induce GM1-lipid gangliosides, which are an integral part of the nervous tissue, with proven neuroprotective effects. It has also been proven that the neuroprotective effect may result from the influence of CQ on sigma-1 receptors [26]

3.2. Cardiotoxicity

In silico methods conducted for cardiotoxicity showed that the drugs are toxic to the heart with high probability. Cardiovascular side effects following CQ/HCQ use include cardiac arrhythmia, vasodilation, and hypotension hypotension [27]. The mechanism of cardiac dysfunction is related to prolongation of the QT interval of the action potential due to blockade of the hERG gene. Repolarization of the action potential is inhibited, which result in blocking the delayed rectifier potassium current (I_{kr}). Molecular modeling

studies have shown that the inhibitory potency value of hERG is IC_{50} = 4.56 μM in the case of CQ racemic form, while for the HCQ racemate it is approx. 3-fold lower (IC_{50} = 12.8 μM)[28]

Additionally, it has been proven that CQ and HCQ influence HCN channels regulating the activity of neurons (HCN1, 2, 4) and cardiomyocytes (HCN3) [29,30]. Consequently, the investigational drugs may affect both myocardial contractility and heart rhythm. It is also worth emphasizing that the literature data confirm the influence of the studied drugs on another channel proposed by CIPA, i.e., $K_{ir}2.1$ channel [29,31]

Previously presented molecular modeling data showed that the inhibitory potency value reflecting the CQ blocking value of I_{K1} is $IC_{50} = 0.69 \pm 0.09$ μM. The data confirm high probability of CQ cardiotoxicity.

4. Conclusion

The combination of preliminary in silico tests with cell-line based methods and validation of results in vitro provides a high probability that the test substance can be used by humans. However, it should be noted that these results cannot be the only way to analyses the compound in terms of safety. The decision to limit the use of CQ and HCQ seems to be correct, because the administration of these drugs may cause neurological and cardiovascular disorders. Nevertheless, it is worth conducting research on the reasonable dosage of the above mentioned preparations, because most of in silico tests do not take into account such parameters as: dose of the substance, the rate of metabolism or elimination of a specific chemical, which are of key in the treatment process. Additional assays, such as molecular modeling, are recommended for a more accurate in silico assessment of interactions between ion channels and test drugs.

However, presented comparison of CQ and HCQ toxicity revealed that the toxicity probability values for both tested drugs have been high. Additionally, CQ is more toxic than HCQ. Considering high neurotoxicity and cardiotoxicity of these drugs it seems peculiar why the FDA's decided to approve them for use during the COVID-19 pandemic so quickly. As the presented research proves even simple in silico tests provide information about serious side effects of using CQ and HCQ.

Conflicts of Interest: The authors declare no conflict of interest.

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