A single dose of *in situ* gel formulation of antimalarial drug chloroquine phosphate as a sustained prophylactic candidate for COVID-19

Noha Talal Zelai
Department of Biological Sciences,
Faculty of Science, King Abdulaziz
University, Jeddah, Saudi Arabia

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Abstract

In the ongoing COVID-19 outbreak, a prophylactic drug is strongly needed to stop the spread of this disease. Chloroquine (CQ) has been proposed as a prophylactic for individuals who are likely to be exposed to the virus. This study aimed to study the ability of CQ to act as a prophylactic treatment for susceptible people. The pharmacokinetic profiles of *in situ* gel and free CQ phosphate were determined using high-performance liquid chromatography. The effects of both formulations were examined on both liver and kidney functions. CQ levels were sustained in the plasma of both free and *in situ* gel-treated groups. Thus, our study shows that the *in situ* gel of CQ provides sustained release of CQ that is given only as a single dose. However, it should be used cautiously in patients with liver or kidney dysfunction.

Key word: Chloroquine phosphate, coronavirus disease 2019, *in situ* gel, pharmacokinetic, prophylactic

INTRODUCTION

According to the September 5, 2020 report published by the World Health Organization, there have been 26,415,380 confirmed cases and 870,286 deaths worldwide because of coronavirus disease 2019 known as COVID-19. The incubation period of this virus ranges from 3 days to 2 weeks. With the maximum incubation period being 14 days, the estimated death rate of COVID-19 is 5.7%.[1] On an average, each patient can infect 2.2 persons, which means that the number of infected cases increases by two times each week. The most vulnerable individuals are health-care providers, the family members of infected patients, and other people who may come into close contact with COVID-19 patients.[2-4]

Recent studies have shown that chloroquine (CQ) can be used for the treatment of COVID-19. CQ has been used as antimalarial drug for 70 years because it plays a role in parasite nucleic acid synthesis and inhibits the parasite's enzymes.[5] It has been reported that CQ increases intralysosomal pH, thus hindering virus-cell fusion, and it plays an inhibitory role in the glycosylation of viral receptors in the host cell.[6] Recent studies have shown that CQ affects the early stages of COVID-19 infection *in vitro*[7] and protects newborn mice from human coronavirus OC43.[8]

It is important to reduce viral transmission and stop the spread of COVID-19 to new victims. To achieve this,

Address for correspondence:
Dr. Noha Talal Zelai,
Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
E-mail: nzelai@kau.edu.sa

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prophylactic measures are urgently needed to protect susceptible people. This prophylaxis is needed to have a sustained release but given as a single dose to improve patient compliance and reduce the pressure on health care providers. There is strong evidence that shows prolonged release and higher levels of drug from an *in situ* gel compared to levels of the free drug.[9,10] In addition, *in situ* gel provides a safe and effective carrier to CQ.[11]

Hence, this study aimed to test the ability of a single dose of *in situ* gel to entrap CQ phosphate and release it in a sustained manner comparing to frequent doses of free CQ that have the same total dose, *in vivo*. In addition, the effects of these formulations on the levels of liver and kidney enzymes were examined.

MATERIALS AND METHODS

Mice and drug formulation

Balb/c mice (n = 30, male and female, mean weight = 25 g) were received from the Breeding Unit of the Animal House in King Saud University, Saudi Arabia. CQ phosphate was gifted by Riyadh Pharma Company in Saudi Arabia.

CQ (50 mg) was first diluted in 1 mL of water by vortexing and water was added to obtain 50 ml of the solution. Fifty milligrams of the drug was dispersed in 50 mg of 30% poly (lactide-co-glycolide) (molecular weight: 24–38 kDa, CAS: 26780-50-7) in *N*-methylpyrrolidone, as described by Tang and Singh.[12] The suspension was vortexed and left for 24–72 h in a 25°C water bath with a shaker.

Mice were randomly distributed into two groups: one group was intramuscularly injected every day for 4 days with 10 mg/kg CQ solution and the second group received one intramuscular inoculum of the gel containing 40 mg/kg CQ. Control group was used to compare liver and kidneys function in normal mice. All experiments granted the approval of the Unit of Biomedical Ethics Research Committee, King Abdulaziz University.

Liver and kidney enzyme tests

Blood samples were taken from mice, 4 days post treatment, by retro-orbital bleeding. Blood plasma was extracted. Urea, creatinine, and uric acid as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase levels in blood plasma were measured using Vitros 350 (Johnson and Johnson, USA).

Euthanasia and anesthesia

Mice were anesthetized by ketamine (90 mg/kg) and xylazine (10 mg/kg), intraperitoneal injection before retro-orbital bleeding and cervical dislocation. Dead mice were placed in biohazardous yellow bags and given to the disposal department in the animal house.

Pharmacokinetics of drug and the main active metabolite

At a wavelength of 260 nm, high-performance liquid chromatography (HPLC) was used to monitor CQ phosphate pharmacokinetics. The mobile phase consists of 30% water contained 1.4 g/l anhydrous dibasic sodium phosphate and 70% methanol contained triethylamine (0.4%) at a flow rate of 1 mL/min and injection volume of 20 μL (USP 2016). Hydroxy CQ (lot # 046M4758V, 25 mg) from Sigma Aldrich (Saint-Louis, Missouri, USA) was used as an internal standard (62.5 μg/mL). To prepare the standards, plasma samples (500 μL) were spiked with serial dilutions of CQ and its metabolite, desethyl CQ (62.5, 31.25, 15.625, 7.8125, 3.90625, and 1.953125 μg/mL).

Blank plasma samples (500 μL) were taken from naïve mice of both sexes and placed in 10 mL tubes. Equivalent concentrations of CQ phosphate and its metabolite (900 μL each) were added to the 10 mL tube. Plasma samples were taken from mice previously injected with 10 mg/kg/dCQ solution for 4 days or 40 mg/kg CQ *in situ* gel at 6, 12, 24, and 48 h after the intraperitoneal injection.

Blood samples from both the mice groups were taken 15 min after the 1st, 2nd, and 3rd doses and on the 5th, 7th, 9th, and 15th day of treatment. *Tert*-butyl methyl ether (5 mL) and NaOH (200 μL, 5 M) were added to each 10 mL tube of plasma sample. The tubes were carefully vortexed for 10 min. This organic phase was transferred into 5 mL tubes after centrifugation at $1500 \times g$ for 10 min, mixed with 0.1 mL HCl (0.1 M) by vortex for 5 min, and centrifuged for 10 min at $1500 \times g$. The upper organic layer was discarded and the bottom aqueous layer was centrifuged again for 20 min at the same speed. Hydroxy CQ (200 μL, 62.5 μg/mL) was added to each vial; standards and samples were then injected into the HPLC.[13]

Statistical analysis

GraphPad Prism software (GraphPad company, San Diego, California, United States of America) was used in drawing all figures and for analyzing all statistical data. All statistical data were calculated as mean ± standard error of mean. One-way ANOVA was used to find the significant relations among data.

RESULTS

Many parameters were used to compare the *in situ* CQ gel and the free CQ formulation.

Kidney and liver function

The levels of kidney and liver enzymes (n = 3) were evaluated to assess the effects of the treated drugs on these enzymes [Figure 1a and b].
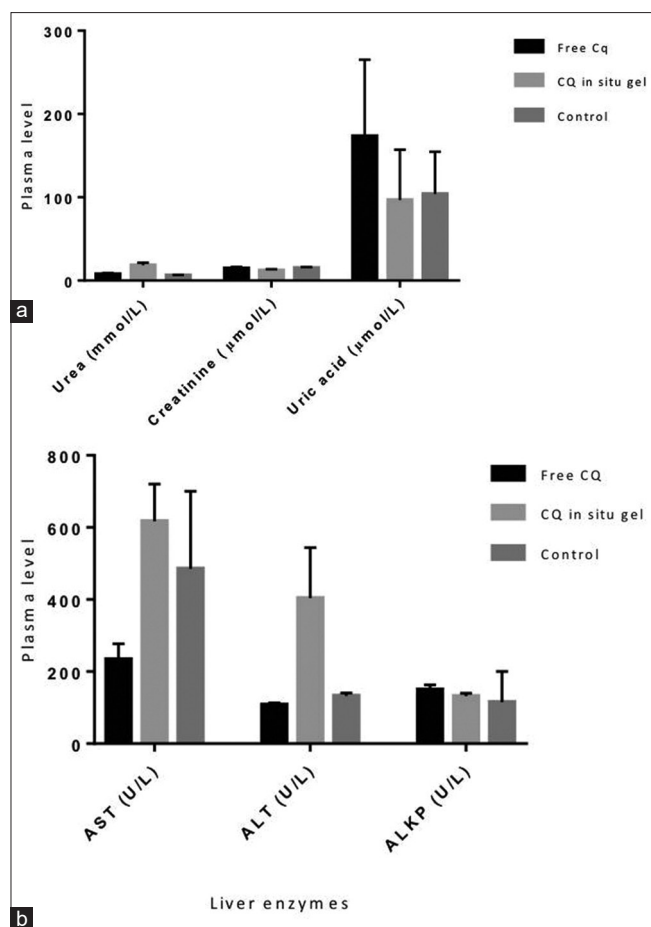
Figure 1: (a) Plasma levels of urea, creatinine, and uric acid. (b) Plasma levels of liver enzymes. Plasma samples were isolated from mouse blood 4 days post infection and tested using Vitros 350. Uric acid was higher in free chloroquine-treated group (although it is not significant). Aspartate aminotransferase and alanine aminotransferase levels were higher in mice that were treated with the *in situ* chloroquine gel. Aspartate aminotransferase; alkaline phosphatase; alanine aminotransferase; chloroquine

Urea levels were significantly higher in CQ *in situ* gel-treated mice ($P = 0.0012$). No statistically significant differences were observed in creatinine or uric acid levels among the groups ($P = 0.0702$ and 0.7787, respectively). Significantly higher AST levels were observed in CQ *in situ* gel-treated and free CQ-treated groups ($P = 0.0393$). No statistically significant differences were found in ALT and Alkaline Phosphatase (ALKP) levels among the groups ($P = 0.1241$ and 0.3430, respectively).

Pharmacokinetics

Plasma concentrations ($n = 3$) of both CQ and its main active metabolite were measured [Figure 2].

No statistically significant differences were detected in the plasma levels of CQ and its main active metabolite between free CQ- and *in situ* gel CQ-treated groups.
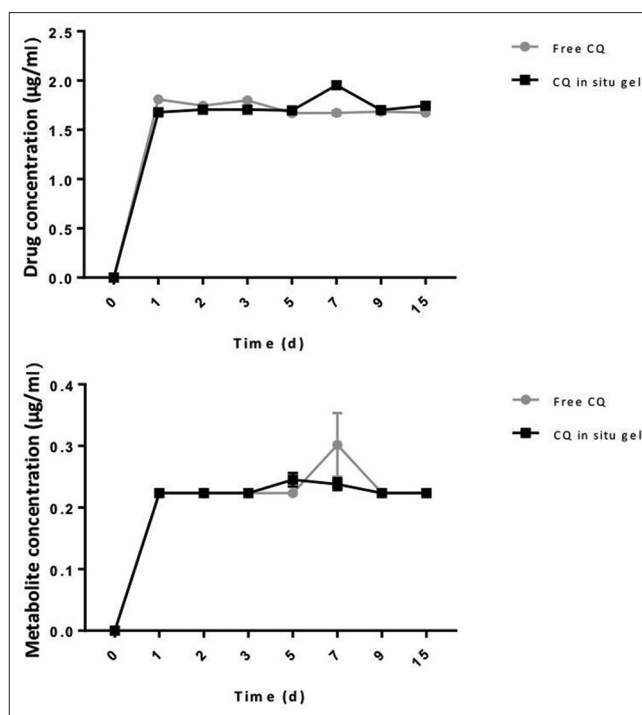

Figure 2: Pharmacokinetic profiles of chloroquine formulations in blood plasma of the treated mice ($n = 3$). The plasma levels of chloroquine and desethyl chloroquine were measured. Both the formulations showed constant plasma levels

DISCUSSION

An initial burst release was observed for both CQ formulations on the 1st day of treatment, and it remained constant for 15 days. Other studies showed that *in situ* gel prolongs the drug release,[9,10] our results support that observation. Thus, the *in situ* gel showed controlled release of CQ that may be useful in the prophylaxis of COVID-19.

Repeated doses of CQ accumulate and get distributed in tissues, such as the liver and kidneys, and have been found to cause the elevation of some enzymes such as alkaline phosphatase;[14] this was observed for the free-drug-treated group, although it was not significant, due to low CQ dose. Other study showed that repeated doses of CQ for 28 weeks cause elevated accumulation of the drug in tissue, mainly in the liver and kidneys.[15]

We observed that CQ elevated the plasma AST levels, which is consistent with the findings of another study.[16] The elevation was only seen in mice treated with the *in situ* gel but not in those treated with free CQ; this suggests that high concentrations of CQ were distributed and sequestered in these organs. The single dose of *in situ* gel CQ raises liver enzyme once the drug was administered; but the repeated dosing causes stead and extended elevation of theses enzymes. Other study showed that *in situ* gel is a safer drug carrier than free drug.[11]

CONCLUSION

CQ *in situ* gel can be a successful candidate to protect COVID-19 infection in susceptible individuals. It has a sustained release that is available for >14 days, which is the maximum incubation period of the disease. It is an efficient formulation that needs to be given only as a single totaled dose, which improves the patient commitment. CQ should not be used by patients who suffer from kidney or liver dysfunction.

Conflicts of interest
There are no conflicts of interest.

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