Chloroquine is effective against influenza A virus in vitro but not in vivo

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Background Chloroquine is an inexpensive and widely available 9-aminoquinolone used in the management of malaria. Recently, in vitro assays suggest that chloroquine may have utility in the treatment of several viral infections including influenza.

Objectives We sought to test whether chloroquine is effective against influenza in vivo in relevant animal models.

Methods The effectiveness of chloroquine at preventing or ameliorating influenza following viral challenge was assessed in established mouse and ferret disease models.

Results Although active against influenza viruses in vitro, chloroquine did not prevent the weight loss associated with influenza virus infection in mice after challenge with viruses expressing an H1 or H3 hemagglutinin protein. Similarly, clinical signs and viral replication in the nose of ferrets were not altered by treatment.

Conclusions Although in vitro results were promising, chloroquine was not effective as preventive therapy in vivo in standard mouse and ferret models of influenza virus infection. This dampens enthusiasm for the potential utility of the drug for humans with influenza.

Key words Influenza, chloroquine, ferret, antiviral.

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Despite the availability of effective antiviral drugs, influenza causes 3–5 million severe illnesses and 250 000–500 000 deaths in the industrialized world annually. There are two classes of drugs currently licensed for use against influenza. The adamantanes, amantadine and rimantadine, target the M2 ion channel, and the neuraminidase inhibitors (NAIs) target the viral sialidase. The adamantane class of drugs has limited effectiveness against currently circulating strains of influenza due to the emergence of resistance. Resistance to the NAIs is not as widespread but is becoming a concern in populations where use is frequent and in the treatment of H5N1 influenza. In addition, NAIs are expensive and are not readily available in many parts of the world. There is therefore intense interest and urgency in the development of new therapeutics that can be implemented for the treatment of influenza.

Recently, a series of in vitro studies have suggested that the antimalarial drug chloroquine may have activity against influenza and other emerging respiratory pathogens. Chloroquine is a lysosomotropic agent that accumulates in the endosomal compartment where it can impair the acidification of the endosome and prevent the conformational changes associated with viral fusion and release into the cytosol. In addition to the effect seen in the endosomal compartment, the drug can also impair viral replication by inhibiting the low-pH-dependent proteases in the Golgi that would participate in glycosylation of nascent viral proteins. Both of these steps are vital for efficient replication and production of viral products. Clinical trials in patients with human immunodeficiency virus 1 (HIV-1) have demonstrated that the addition of chloroquine to existing treatment protocols can decrease the production of infectious particles at doses in the physiologic range for malaria. This use of chloroquine in HIV-1 patients has led some authors to speculate on its potential utility in the treatment of other viral infections. If chloroquine were found to be efficacious in vivo, its use would have several attractive features including a unique mechanism of action, lack of cross-resistance with other antiviral drugs, low cost, and widespread worldwide availability.

The goal of this study was to determine whether chloroquine could decrease morbidity from influenza virus infection in two relevant animal models, the mouse and the ferret. Two viruses were utilized in mice. The mouse-adapted Mount Sinai strain of A/Puerto Rico/8/34 (PR8) is an H1N1 subtype virus taken from the influenza virus repository at St. Jude Children’s Research Hospital. To determine whether findings were subtype specific, a second
virus was generated using the eight plasmid reverse genetics system, which contains the H3 hemagglutinin (HA) and N2 neuraminidase (NA) from A/Hong/Kong/1/68 (HK68; H3N2) and the six internal genes of PR8 as described. The PR8 backbone was utilized to enhance virulence in mice as the wild-type parent HK68 causes only mild disease and is not lethal. Both viruses were grown in Madin-Darby canine kidney (MDCK) cells. Before conducting mouse studies, the susceptibility of these viruses to chloroquine in vitro was assessed. A549 cells were infected at a multiplicity of infection of 0.1 with viruses for 48 hours in the presence or absence of 5, 10, or 20 μM of chloroquine. Cells were collected 48 hours post-infection, stained with Alexa-488 anti-HA and surface HA expression was assessed by flow cytometry. As has been reported previously, chloroquine was effective in vitro, reducing the percentage of cells expressing HA by 56–79% at a 20 μM concentration compared to no treatment, with lesser reductions at 5 and 10 μM (data not shown).

To determine whether chloroquine was effective in vivo, we tested the drug in an established mouse model of infection using age and weight matched groups of 8- to 24-week-old female Balb/c mice. Chloroquine was given daily starting 24 hours prior to infection with dosing based on prior published protocols evaluating its use protecting daily starting 24 hours prior to infection with dosing based on prior published protocols evaluating its use protecting

![Figure 1. Effect of chloroquine (CQ) on influenza virus infection in mice. (A) Groups of nine mice were infected with influenza viruses PR8 (H1N1) or HK68 (H3N2), dosed with 12.5 mg/kg/day of chloroquine starting 24 hours prior to infection, and followed for weight loss (mean ± SD) compared to mock-treated animals. (B) Groups of five mice were infected with HK68, dosed with either 25 or 37.5 mg/kg/day of chloroquine starting 24 hours prior to infection, and followed for weight loss compared to mock-treated animals. An asterisk (*) indicates a significant difference in weight loss compared to the other groups at that time point (P < 0.05 by ANOVA).](image-url)
chloroquine appeared to have little effect on influenzal infection in ferrets.

In conclusion, we have found that although chloroquine is effective in vitro at limiting the replication of viruses expressing either an H1 or H3 HA in concurrence with other published reports,\(^7\),\(^8\) this effect does not extend to in vivo models of influenza. At a concentration of 12.5 mg/kg/day we were not able to protect mice from significant weight loss following infection with either HK68 or PR8. At higher concentrations, the effect of viral infection was enhanced and treated mice lost more weight than mock-treated animals. Studies exploring the prophylactic effect of chloroquine in a ferret model system that more closely matches human infection likewise did not demonstrate any significant positive effects. It is possible that higher doses of chloroquine or analogs such as hydroxychloroquine with potentially better pharmacokinetics may have a beneficial effect that we were unable to elicit in our models. Studies of combination therapy with current anti-influenza drugs and chloroquine may also be of use in developing more effective therapies for the treatment and prophylaxis of influenza. However, confirmation of the findings of this study in other laboratories is likely to significantly dampen enthusiasm for use of chloroquine as an antiviral against influenza.

**Conflicts of interest**

The authors declare that they have no conflicts of interest.

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