Chloroquine for influenza prevention: a randomised, double-blind, placebo controlled trial

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Summary

Background **Chloroquine has in-vitro activity against influenza and could be an ideal candidate for worldwide prevention of influenza in the period between onset of a pandemic with a virulent influenza strain and the development and widespread dissemination of an effective vaccine. We aimed to assess the efficacy of such an intervention.**

Methods **In this randomised, double-blind, placebo-controlled trial done at a single centre in Singapore, we randomly assigned (1:1) healthy adults to receive chloroquine phosphate (500 mg/day for 1 week, then once a week to complete 12 weeks) or matching placebo by use of a computer-generated randomisation list. Participants filled an online symptom diary every week, supplemented by daily diaries and self-administered nasal swabs when unwell. Haemagglutination-inhibition assays for influenza A (H1N1, H3N2) and B were done on blood samples taken at baseline and after 12 weeks. The primary outcome was laboratory-confirmed clinical influenza defined by specific symptoms accompanied by influenza RNA on nasal swabs or a four-fold increase in haemagglutination-inhibition titres over the 12-week study period. Analysis was by intention to treat. This trial was registered with ClinicalTrials.gov, number NCT01078779.**

Findings **From November, 2009, to February, 2010, we recruited 1516 eligible participants. 1496 (96%) returned at week 12 and were included in the efficacy analysis. Adherence to study intervention was 97%, and 94% of the scheduled weekly diaries were completed. Eight (1%) of 738 participants had laboratory-confirmed clinical influenza in the placebo group and 12 (2%) of 724 in the chloroquine group (relative risk 1·53, 95% CI 0·63–3·72; p=0·376). 29 (4%) of 738 had laboratory-confirmed influenza infection (symptomatic or asymptomatic) in the placebo group and 38 (5%) of 724 in the chloroquine group (1·34, 0·83–2·14; p=0·261). 249 (33%) of 759 participants reported adverse events (mostly mild) in the placebo group and 341 (45%) of 757 in chloroquine group (p<0·0001). Headache, dizziness, nausea, diarrhoea, and blurred vision were more common in the chloroquine group, but rarely resulted in treatment discontinuation. One serious adverse event (hepatitis) was possibly related to chloroquine.**

Interpretation **Although generally well tolerated by a healthy community population, chloroquine does not prevent infection with influenza. Alternative drugs are needed for large-scale prevention of influenza.**

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See Comment page 653

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Introduction

The rapid spread and magnitude of the 2009 H1N1 influenza pandemic shows clearly that the current armamentarium available for prevention of global influenza transmission is insufficient. Vaccine production often takes more than 6 months from identification of a new strain to widespread dissemination to the public, by which time the initial wave of a pandemic is likely to have passed.[1] Neuraminidase inhibitors are effective in pre-exposure prophylaxis of influenza,[2] but such use of these drugs on a large-scale, long-term basis is not feasible for many reasons, including insufficient manufacturing capacity, enormous cost, and risk of promoting resistance that would impair their efficacy in the treatment of influenza. Even with adequate supply of vaccines and drugs, many additional challenges to prevention of pandemic influenza transmission in developing countries exist—especially limitations of health-care infrastructure and human resources.[3] A simple, well tolerated, internationally accessible, and economical pharmacological intervention for the prevention of influenza could avert millions of deaths during the period between onset of a virulent influenza pandemic and the development and worldwide dissemination of an effective vaccine.

Chloroquine, an antimalarial drug that has been in widespread clinical use for more than 50 years, has broad-spectrum antiviral activity because it increases endosomal pH, which disrupts pH-dependent structural changes in viral-synthesised proteins.[4] Chloroquine has in-vitro activity against both H1N1 and H3N2 influenza strains at concentrations that are achievable in vivo at the doses of chloroquine used for malaria prophylaxis and treatment of connective-tissue diseases.[5,6] Although chloroquine did not prevent influenza infection or diminish the weight loss in mice infected with a mouse-adapted H1N1 strain, it did show some effectiveness in limiting viral replication in ferrets infected with an adapted H3N2 strain.[7] It might be an ideal drug for worldwide pre-exposure prevention of influenza. Chloroquine can be taken once a week, is well tolerated and safe (including use in pregnant women and children), is available in sufficient quantities

worldwide for an immediate roll-out programme if effectiveness were to be established, is cost effective for use in resource-poor countries, and has a putative mechanism of action that is unlikely to be compromised by antiviral resistance. Several expert reviews have called for an assessment of generic drugs, such as chloroquine, for prevention of influenza.[8,9]

No trials of chloroquine for the prevention of influenza have been done in man, but in view of its antiviral properties and overall suitability as a prophylactic drug, we postulated that it might be of value. Furthermore, the drug also has anti-inflammatory and immunomodulatory actions that might be beneficial for prevention of progression to severe disease, since the pathogenesis of severe influenza is driven by an inflammatory immune response to the infection.[8,10] We aimed to assess whether chloroquine, taken once a week by healthy individuals in the community during a pandemic, would protect against infection with influenza or decrease the severity of influenza symptoms.

Methods

Study design and participants

The chloroquine for influenza prevention (CHIP) trial was a randomised, double-blind, placebo-controlled clinical trial done at a single site (the Investigational Medicine Unit, National University Health System, Singapore). Participants were recruited between November, 2009, and February, 2010, through a study website that provided information about the trial and an opportunity to register for a screening visit. The website address was advertised around the university campus and hospital and widely in the national media. Follow-up was completed in May, 2010.

Eligible participants were male or female healthy volunteers aged 18–65 years. Exclusion criteria were a history of psoriasis, porphyria cutanea tarda, epilepsy, myasthenia gravis, myopathy of any cause, cardiac arrhythmias, retinal disease, serious hepatic or renal disease, known glucose-6-phosphate dehydrogenase deficiency, current use of medication with known serious hepatotoxic effects or known interaction with chloroquine, severe depression, influenza vaccination within the past 3 months, acute clinical influenza at screening, and pregnancy or breastfeeding.

For the **CHIP trial protocol** see http://www.nuhs.edu.sg/wbn/slot/u2934/CHIP_protocol_V2_6%20clean.pdf

The trial protocol was approved by the ethics committee of the National Healthcare Group and all participants provided written informed consent.

Randomisation and masking

The trial statistician prepared a computer-generated randomisation list with a 1:1 ratio and a prepared block size of ten. A pharmacist, independent of the trial team, placed labels with four-digit study identifiers on bottles of placebo and chloroquine according to the randomisation list. Randomisation was done by computer after baseline data had been gathered. Participants were individually randomised (ie, any household association with other participants was ignored). All trial investigators, clinical and laboratory staff, and participants were masked to the treatment allocation. A procedure for emergency unblinding of individual participants was established, although no unblinding was necessary during the trial.

Procedures

Demographic characteristics and a clinical history were recorded at baseline, and height, weight, and vital signs were measured. Blood was drawn for storage of serum. Both study groups received identical capsules containing either chloroquine phosphate 250 mg (equivalent to 150 mg base) or lactose placebo (Beacons Pharmaceuticals, Singapore). Participants were instructed to take two capsules once a day with food for the first 7 days of the trial, starting on the day after randomisation. After this induction period, they took two capsules once a week, on a designated day of the week to complete a total course of 12 weeks. Participants were telephoned at the end of the first week to check adherence to the study intervention, and to record any side-effects.

Medical management of participants with influenza or other illnesses was provided through the usual community channels, and health-care providers were asked to complete forms with details of diagnosis and any diagnostic tests done to identify influenza at each clinic visit. If the participant decided to receive an influenza vaccination during follow-up they were asked to return to the trial site to have an additional blood draw before having the vaccination.

During follow-up, participants completed weekly and daily diaries on a secure, password-protected trial website. Weekly diaries were completed on the day that was designated for taking study treatment, and included information about symptoms, visits to health-care workers, and adherence to the study intervention. Participants received reminder messages each week via email and mobile phone text message. They completed a daily diary if they developed any influenza-like symptoms, and continued this diary until the symptoms resolved. This diary consisted of a checklist of symptoms, which participants graded as none, mild, or moderate to severe, and a report of other symptoms, body temperature (a standard digital thermometer was provided), and absence from work or school caused by sickness.

Participants were given a pack containing two nasal swabs and an RNA-stabilising compound (PrimeStore, Longhorn Vaccines and Diagnostics, San Antonio, USA) and were instructed to take a swab from each nostril on the second day of any illness associated with fever, runny nose, or cough. After use, swabs were sealed and kept in the refrigerator until they were returned at the week 12 visit. Participants called the trial hotline after they had used the swabs, and a replacement pack was sent by post within 1 week for use during any subsequent symptomatic episode. Pretrial testing showed that influenza RNA did

not degrade in PrimeStore media after 30 days, even when maintained at 37°C.

The second trial visit was at 12 weeks (window 3 days before to 21 days after) from the baseline visit. Adherence to study intervention was assessed by self-report of missed doses and by pill count. We obtained a participant history of influenza vaccination, influenza-like illness, rash or itch, and any other clinical events occurring since the baseline visit. Adverse events were graded with standard toxicity criteria,[11] and a blood sample was drawn.

We tested all paired serum samples with haemagglutination-inhibition assays to detect infection with influenza H1N1, H3N2, or influenza B with standard laboratory methods.[12] We did these assays with influenza viruses isolated from local patients presenting with acute febrile illness to primary health-care clinics in 2009. Infection was defined as at least a four-fold increase in antibody titre from baseline to week 12. We retested specimens with a borderline increase, which did not meet diagnostic criteria, by use of a microneutralisation assay with a previously described method.[13] Nasal swabs were tested by RT-PCR for influenza virus with a method that targets the M gene of the virus;[14] additional testing was done with primers specific to the pandemic H1N1 strain.[15]

The primary endpoint was laboratory-confirmed clinical influenza, defined as the combination of symptoms of clinical influenza and laboratory-confirmed influenza infection—a standard definition used in influenza prevention trials.[16,17] We defined clinical influenza as a reported temperature of at least 37·2°C, with at least one respiratory symptom (sneezing, runny nose, blocked nose, sore throat, dry cough, coughing up phlegm, wheezing, shortness of breath) and at least one constitutional symptom (feeling feverish, muscle aches, fatigue, headache, diarrhoea) occurring on the same day. Laboratory-confirmed influenza infection was defined as one of the following test results: (1) PCR confirmation of influenza on a nasal swab taken by the participant; (2) PCR confirmation or culture for influenza obtained from a nasopharyngeal swab taken by a health-care practitioner in the community; (3) serological confirmation by at least a four-fold increase in antibody titre on haemagglutination-inhibition or micro-neutralisation assay for H1N1, H3N2, or influenza B infection from baseline to week 12. For participants who had H1N1 influenza vaccination during follow-up, the change in H1N1 titre at week 12 was disregarded, but the change in H1N1 titre at a prevaccination follow-up blood sampling was included in the analysis if available. We used a similar approach for participants who received seasonal influenza vaccination during the study.

The main secondary endpoint was laboratory-confirmed influenza infection, whether symptomatic or asymptomatic (ie, without the requirement for clinical influenza symptoms). In those with laboratory-confirmed influenza infection, we also assessed outcomes associated with severity of illness: symptomatic clinical influenza; severe symptomatic clinical influenza (but with a temperature ≥37·8°C and at least five moderate to severe clinical influenza symptoms occurring on the same day); maximum number of symptoms of clinical influenza that were graded as moderate to severe at the peak-illness day; presence of individual constitutional symptoms (feeling feverish, muscle aches, fatigue, headache, diarrhoea) that were graded as moderate to severe; and the number of days off school or work due to clinical influenza.

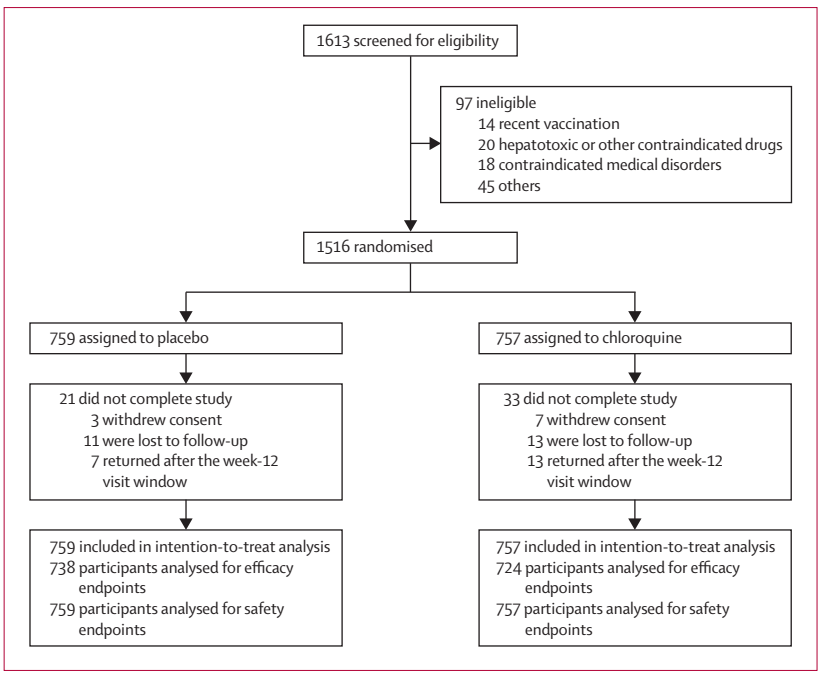

***Figure*: Trial profile**

Statistical analysis

We estimated that the rate of laboratory-confirmed clinical influenza would be at least 10% over the 12-week study period, consisting of 5% incidence of seasonal influenza (based on Singapore national data[18] and data from an influenza-prevention trial in the USA),[16] and a 5% incidence of H1N1 pandemic influenza infection, which was considered to be a conservative estimate in view of the much higher transmissibility of the H1N1 strain than that of seasonal influenza.[19] We reasoned that a 50% reduction in clinical cases would justify widespread clinical use of chloroquine and would be a realistic effect size in view of the 74% reduction seen with pre-exposure prophylaxis with oseltamivir.[16] We calculated that a sample size of 1500 participants (750 per group) would give a power of 90% to detect a 50% reduction in incidence of the primary endpoint at the 5% significance level (adjusted for 10% loss to follow-up and for household correlation, assuming at most two participants per household and an intraclass correlation coefficient of 0·1).

Primary efficacy analyses were done on an intention-to-treat basis, including all eligible participants who were randomised and who returned for a week-12 visit within the specified window period. A supplementary analysis was done on a per-protocol dataset that was based on the intention-to-treat population, but excluded participants who delayed starting the study intervention for more than 7 days after randomisation, or who took less than 50% of the loading dose or less than 25% of the maintenance dose. Safety analysis included all eligible randomised participants. Analysis of indicators of severity of illness was done on the subset of those with laboratory-confirmed influenza infection.

We calculated relative risks with 95% CIs and two-sided p values using the exact method.[20] Analyses were done on the basis of individual participants (as randomised), without taking into account the effects of clustering. The number of symptoms graded as moderate to severe and the number of days off school or work due to clinical influenza were compared between the two groups by Wilcoxon rank-sum tests. This trial was registered with ClinicalTrials.gov, number NCT01078779.

	Placebo (n=759)	Chloroquine (n=757)
Male sex	486 (64%)	472 (62%)
Age (years)	23·5 (22·1–31·6)	23·6 (22·1–32·0)
Age group		
18–24	478 (63%)	475 (63%)
25–34	122 (16%)	126 (17%)
35–44	88 (12%)	71 (9%)
45–54	53 (7%)	63 (8%)
55–64	18 (2%)	22 (3%)
Ethnic origin		
Chinese	618 (81%)	604 (80%)
Indian	56 (7%)	55 (7%)
Malay	39 (5%)	53 (7%)
Other	46 (6%)	45 (6%)
Place of work or study		
Child-care facility or primary school	3 (<1%)	4 (<1%)
Secondary school, college, or university	481 (63%)	474 (63%)
Health-care facility	52 (7%)	49 (6%)
Other	223 (29%)	230 (30%)
Smoker	76 (10%)	62 (8%)
Body-mass index (kg/m²)	22·3 (20·2–24·9)	22·4 (20·4–24·8)

Data are number of participants (%) or median (IQR).

Table 1: **Baseline characteristics**

	Placebo (n=738)	Chloroquine (n=724)	Relative risk (95% CI)	p value
Primary endpoint				
Laboratory-confirmed clinical influenza	8 (1%)	12 (2%)	1·53 (0·63–3·72)	0·376
Main secondary endpoint				
Laboratory-confirmed influenza infection	29 (4%)	38 (5%)	1·34 (0·83–2·14)	0·261
Other secondary endpoint				
Clinical influenza	63 (9%)	57 (8%)	0·92 (0·65–1·30)	0·703

Data are number of participants (%) or relative risk (95% CI).

Table 2: **Efficacy analysis.**

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

Results

Of 1516 eligible participants enrolled and randomly assigned to study treatment, 1462 (96%) returned for the week-12 visit (figure). Table 1 shows demographic and clinical characteristics for the two study groups. Adherence to study intervention was high (97% doses taken) and only 31 participants reported discontinuation (ten in the placebo group, 21 in the chloroquine group; p=0·048). 31 (2%) participants received influenza vaccination during the trial follow-up (16 in the placebo group and 15 in the chloroquine group; 23 received H1N1 vaccinations, five seasonal, and three both).

A total of 17 521 weekly diaries (94% of those scheduled) and 4037 elective daily symptom diaries were completed. 120 (8%) participants reported one or more episodes of symptoms that met the definition of clinical influenza. There were 60 laboratory-confirmed influenza infections detected by haemagglutination-inhibition testing. Nasal swabs were taken in 43% of symptomatic episodes. A total of 210 nasal swabs were obtained, from which 13 cases of influenza were detected (six were confirmed

	Placebo (n=29)	Chloroquine (n=38)	Relative risk (95% CI)	p value
Clinical influenza	8 (28%)	12 (32%)	1·14 (0·54–2·43)	0·792
Severe clinical influenza	1 (3%)	1 (3%)	0·76 (0·05–11·69)	0·999
Asymptomatic influenza infection	15 (48%)	18 (47%)	0·98 (0·59–1·62)	0·999
Number of days off school, college, or work due to clinical influenza	0 (0–2)*	0 (0–2)†	..	0·486
Number of moderate to severe symptoms at the peak day of clinical-influenza symptoms	0 (0–7)‡	0 (0–6)§	..	0·952

Data are number of participants (%), relative risk (95% CI), or median (range). *n=23. †n=33. ‡n=26. §n=37.

Table 3: **Analysis of clinical disease severity in participants with laboratory-confirmed influenza infection**

	Placebo (n=759)	Chloroquine (n=757)	p value
One or more adverse events	249 (33%)	341 (45%)	<0·0001
Adverse events with frequency ≥5% in any group			
Headache	55 (7%)	119 (16%)	<0·0001
Dizziness	12 (2%)	72 (10%)	<0·0001
Sore throat	69 (9%)	68 (9%)	0·999
Nausea	8 (1%)	59 (8%)	<0·0001
Cough	58 (8%)	52 (7%)	0·621
Diarrhoea	26 (3%)	42 (6%)	0·048
Running nose	30 (4%)	38 (5%)	0·324
Other adverse events			
Itching	16 (2%)	28 (4%)	0·068
Blurred vision	2 (<1%)	23 (3%)	<0·0001
Adverse events leading to treatment discontinuation	7 (1%)	13 (2%)	0·186
One or more serious adverse events	5 (1%)	3 (<1%)	0·726

Data are number of participants (%).

Table 4: **Adverse events**

by serology, seven by PCR alone; 11 were H1N1). No additional laboratory-confirmed infections were identified by microneutralisation tests or by tests done in the community. Of 67 laboratory-confirmed infections, 51 were identified as H1N1 infection alone, six as H3N2 infection alone, eight as influenza B infection alone, and two were mixed. By combination of clinical and laboratory diagnostic criteria, 20 (1%) participants met the primary-endpoint definition of laboratory-confirmed clinical influenza.

The primary endpoint of laboratory-confirmed clinical influenza and secondary endpoint of laboratory-confirmed influenza infection, whether symptomatic or asymptomatic, did not differ between groups (table 2). We did not identify a significant difference between the two treatment groups for the number of clinic visits or admissions to hospital (data not shown). Results were similar when the analysis was repeated, taking into account the effects of clustering, and when repeated with the 1431 participants in the per-protocol dataset (data not shown).

We recorded no significant difference between the groups in clinical severity of influenza (various definitions) in the 67 participants who had laboratory-confirmed influenza infection (table 3).

More participants in the chloroquine group reported adverse events than in the placebo group (table 4), although these events were judged as mild (or grade 1) in almost all cases. Headache, dizziness, nausea, and diarrhoea were more common in the chloroquine group, but rarely resulted in treatment discontinuation (table 4).

Eight serious adverse events were reported, five in the placebo group (haematemesis, renal stone, acute appendicitis, ankle fracture, prolapsed intervertebral disc) and three in the chloroquine group (maxillary cyst, hepatitis, endometrial carcinoma). The only event in the chloroquine group judged to be possibly related to the study drug was symptomatic hepatitis starting about 5 weeks after initiation of chloroquine and 10 days after the participant returned from a holiday on a nearby tropical island. Concentrations of hepatic transaminases were raised to more than ten times the upper limit of normal at presentation, and returned to normal 1 month after discontinuation of the study intervention. The hospital discharge diagnosis was viral hepatitis, but standard viral serology tests were negative.

Discussion

We did not find evidence that chloroquine, when given in a malaria prophylaxis dose, had protective efficacy against laboratory-confirmed clinical influenza or laboratory-confirmed influenza infection (symptomatic or asymptomatic) in this adult population (panel).

We designed this trial to detect a level of protective efficacy of chloroquine of at least 50%, which is the minimum level of protection that we thought would be necessary to show for chloroquine to be accepted as a worthwhile global intervention for prevention of influenza transmission during a pandemic. Lower levels of protection might be of academic interest, but would be unlikely to lead to widespread acceptance as a public health intervention in a pandemic situation because of the probable diminished effectiveness of the intervention in a real-life setting (as opposed to a clinical trial), the possibility of individual risk compensation (ie, decreased compliance with other prevention measures because of perceived high-level protection from pharmacological prophylaxis), and the possibility of competition for scarce health-care-infrastructure resources, which might impede the roll-out of other influenza risk-reducing strategies.

Although continuing local transmission of pandemic H1N1 strain influenza in Singapore (along with increasing H3N2 and B strain transmission) during follow-up was substantial, it did not yield the number of clinical influenza infections that we had anticipated—partly because the transmission had declined after the peak of the pandemic and because many of the infections that occurred were asymptomatic. As a result, estimates of protective efficacy

Panel: **Research in context**

Systematic review

We searched PubMed from inception to March 7, 2011, with the terms "chloroquine" OR "hydroxychloroquine" AND "influenza" AND "prevention" OR "treatment"; no langauge restrictions were used. We identified no previous clinical trials of chloroquine in the prevention or treatment of influenza.

Interpretation

We showed that chloroquine taken once weekly does not have protective efficacy against influenza infection, and does not appear to diminish the symptoms of established infection. An effective drug is needed that can be used internationally on a large scale for the prevention of influenza.

of chloroquine that we obtained were imprecise. Nevertheless, even with the most optimistic estimates from the 95% CI, we showed that chloroquine has no more than 37% protective efficacy against laboratory-confirmed clinical influenza and no more than 17% protective efficacy against the more precise measure of laboratory-confirmed infection (symptomatic or asymptomatic). Thus, according to our a-priori criterion for efficacy, the results of this study rule out a useful role of chloroquine as a general intervention to prevent pandemic influenza transmission. Furthermore, the point estimate of efficacy that we obtained suggests that chloroquine might actually increase the risk of influenza infection, although we believe that this increased risk is probably a chance finding. However, in view of the diversity of immunological actions of chloroquine, it might increase the risk of influenza infection through some as yet unknown pathway.

Several possible reasons exist as to why the efficacy of chloroquine against influenza shown in vitro did not translate to efficacy in vivo.[5,6] First, influenza strains vary in their pH requirements for viral entry and hence susceptibility to chloroquine, and efficacy of chloroquine against the H1N1 pandemic strain might have been low (the predominant strain in circulation during the trial). However, in-vitro studies have shown efficacy of this drug against H1N1 infection,[5] and our trial provided no evidence of an effect of chloroquine against non-H1N1 infections either, so this explanation seems unlikely. Our results are consistent with findings from studies of dengue infection in which the in-vitro effects of chloroquine did not translate into in-vivo efficacy,[21] although they have done so in other viral infections, especially HIV infection.[22–27]

Second, the possibility of bias should be considered. Although chloroquine is easily recognised by its bitter taste, this taste was masked effectively by formulating the drug in capsules. The use of blinding and the high rates of completion of symptom diaries and the high proportion of participants who returned for follow-up visits make significant bias unlikely to account for the result. Furthermore, chloroquine was not effective against laboratory-confirmed influenza infection, an entirely objective outcome variable.

Third, despite very high levels of adherence to study intervention, tissue levels of chloroquine might have been inadequate to protect against influenza viral entry. The choice of chloroquine dose for this trial was pragmatic. We took into account the high degree of convenience and tolerability that would be needed for chloroquine, if shown to be effective, to gain widespread acceptance as an influenza pre-exposure prophylaxis regimen taken for many months by healthy members of the community, and the safety factors that make it acceptable to give a dose of chloroquine each week (but not a sustained high dose given every day) without undertaking regular laboratory monitoring. In view of the long half-life of chloroquine, we calculated that the dose given each week should maintain blood concentrations achieved in the once-a-day induction phase[28] and should be roughly the half maximal inhibitory concentration (IC50) of the H1N1 and H3N2 strains used in the in-vitro studies. Furthermore, the drug is highly concentrated in many tissues with sustained use including the lungs, and in white cells and macrophages, and such intracellular concentrations might be more relevant for activity of the drug against influenza viral entry.[5,6,28,29] At the dose given, headache, dizziness, nausea, diarrhoea, and blurred vision were significantly more common in participants taking chloroquine than in those taking the placebo, although these were mainly transient during the first week of treatment, and rarely led to treatment discontinuation. Although a higher dose of chloroquine could have been sustained for a longer period, despite these side-effects, this dose would probably have restricted the acceptability of the intervention for many people. The case of symptomatic hepatitis, which was possibly related to chloroquine (although symptomatic hepatitis is rare with this drug), reinforces the belief that sustained higher doses would need systematic laboratory monitoring, which would seriously limit the generalisability of the intervention. Thus, although we cannot rule out efficacy at a higher dose of chloroquine than the one we tested, we believe that the tolerability and safety findings in this study confirm that the dose selected was appropriate for an intervention to prevent influenza that would be generalisable worldwide.

Chloroquine has anti-inflammatory properties that might help to stop the virus-induced inflammation that drives disease pathogenesis after the first few days.[8,10] Treatments directed against the host response to influenza are notable, because they might have the potential to decrease the mortality associated with severe influenza at a stage when antiviral drugs are ineffective. However, we saw no evidence of a difference in severity of disease in people who acquired influenza on chloroquine or placebo that might support this notion.

For short-term treatment of influenza, high doses of chloroquine given every day could possibly be used, and in view of the low efficacy of current influenza treatments this approach might still be worth testing. Furthermore, absence of evidence of benefit from chloroquine monotherapy does not preclude the possibility of activity when used in combination with standard antiviral drugs, and this might merit further investigation.

We studied healthy volunteers, most of whom were Chinese. Although our finding that chloroquine does not have protective efficacy against influenza infection is probably generalisable to other populations, its effects on the host immune response to influenza, and hence disease severity, might be greater in an older population with more comorbidity, or in different ethnic groups.

An effective, well-tolerated, cheap, and widely available prophylactic drug is needed that can be used as a part of

an international public health response to influenza. Identification of this compound needs to be done with some urgency before a pandemic occurs (or recurs) with a virulent strain of influenza virus.

Contributors

NIP designed the trial, led the trial as the chief investigator, and wrote the final report. LL was the principal investigator of the trial, with overall responsibility for the conduct of the trial at the site, and for medical oversight of trial implementation. GW was responsible for the operational aspects of trial implementation. SA contributed to the clinical and operational implementation of the trial. YX and YBC were responsible for analysing the data. EEO was responsible for the laboratory analyses. AW-S was responsible for recruitment of participants. All authors contributed to trial design and interpretation of data and reviewed the final report.

Conflicts of interest

We declare that we have no conflicts of interest.

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References

1 Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. *Nature* 2006; **442:** 448–52.

2 Jefferson T, Jones M, Doshi P, Del Mar C, Dooley L, Foxlee R. Neuraminidase inhibitors for preventing and treating influenza in healthy adults. *Cochrane Database Syst Rev* 2010; **2:** CD001265.

3 Oshitani H, Kamigaki T, Suzuki A. Major issues and challenges of influenza pandemic preparedness in developing countries. *Emerg Infect Dis* 2008; **14:** 875–80.

4 Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis* 2003; **3:** 722–27.

5 Ooi EE, Chew JS, Loh JP, Chua RC. In vitro inhibition of human influenza A virus replication by chloroquine. *Virol J* 2006; **3:** 39.

6 Di Trani L, Savarino A, Campitelli L, et al. Different pH requirements are associated with divergent inhibitory effects of chloroquine on human and avian influenza A viruses. *Virol J* 2007; **4:** 39.

7 Vigerust DJ, McCullers JA. Chloroquine is effective against influenza A virus in vitro but not in vivo. *Influenza Other Respi Viruses* 2007; **1:** 189–92.

8 Fedson DS. Confronting an influenza pandemic with inexpensive generic agents: can it be done? *Lancet Infect Dis* 2008; **8:** 571–76.

9 Fedson DS. Meeting the challenge of influenza pandemic preparedness in developing countries. *Emerg Infect Dis* 2009; **15:** 365–71.

10 de Jong MD, Simmons CP, Thanh TT, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006; **12:** 1203–07.

11 Division of AIDS N. Division of AIDS table for grading severity of adult adverse experiences. Rockville: National Institute of Allergy and Infectious Diseases, 1992.

12 WHO. WHO manual on animal influenza diagnosis and surveillance. Geneva: World Health Organization, 2002.

13 Rowe T, Abernathy RA, Hu-Primmer J, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. *J Clin Microbiol* 1999; **37:** 937–43.

14 Fouchier RA, Bestebroer TM, Herfst S, Van Der Kemp L, Rimmelzwaan GF, Osterhaus AD. Detection of influenza A viruses from different species by PCR amplification of conserved sequences in the matrix gene. *J Clin Microbiol* 2000; **38:** 4096–101.

15 Wang R, Sheng ZM, Taubenberger JK. Detection of novel (swine origin) H1N1 influenza A virus by quantitative real-time reverse transcription-PCR. *J Clin Microbiol* 2009; **47:** 2675–77.

16 Hayden FG, Atmar RL, Schilling M, et al. Use of the selective oral neuraminidase inhibitor oseltamivir to prevent influenza. *N Engl J Med* 1999; **341:** 1336–43.

17 Welliver R, Monto AS, Carewicz O. Effectiveness of oseltamivir in preventing influenza in household contacts: a randomized controlled trial. *JAMA* 2001; **285:** 748–54.

18 Ng TP, Pwee KH, Niti M, Goh LG. Influenza in Singapore: assessing the burden of illness in the community. *Ann Acad Med Singapore* 2002; **31:** 182–88.

19 Fraser C, Donnelly CA, Cauchemez S. Pandemic potential of a strain of influenza A (H1N1): early findings. *Science* 2009; **324:** 1557–61.

20 Greenwood B. Interpreting vaccine efficacy. *Clin Infect Dis* 2005; **40:** 1519–20.

21 Tricou V, Minh NN, Van TP, et al. A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. *PLoS Negl Trop Dis*; **4:** e785.

22 Chiang G, Sassaroli M, Louie M, Chen H, Stecher VJ, Sperber K. Inhibition of HIV-1 replication by hydroxychloroquine: mechanism of action and comparison with zidovudine. *Clin Ther* 1996; **18:** 1080–92.

23 Sperber K, Kalb TH, Stecher VJ, Banerjee R, Mayer L. Inhibition of human immunodeficiency virus type 1 replication by hydroxychloroquine in T cells and monocytes. *AIDS Res Hum Retroviruses* 1993; **9:** 91–98.

24 Savarino A, Gennero L, Chen HC, et al. Anti-HIV effects of chloroquine: mechanisms of inhibition and spectrum of activity. *AIDS* 2001; **15:** 2221–29.

25 Vincent MJ, Bergeron E, Benjannet S, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol J* 2005; **2:** 69.

26 Sperber K, Chiang G, Chen H, et al. Comparison of hydroxychloroquine with zidovudine in asymptomatic patients infected with human immunodeficiency virus type 1. *Clin Ther* 1997; **19:** 913–23.

27 Sperber K, Louie M, Kraus T, et al. Hydroxychloroquine treatment of patients with human immunodeficiency virus type 1. *Clin Ther* 1995; **17:** 622–36.

28 Wetsteyn JC, De Vries PJ, Oosterhuis B, Van Boxtel CJ. The pharmacokinetics of three multiple dose regimens of chloroquine: implications for malaria chemoprophylaxis. *Br J Clin Pharmacol* 1995; **39:** 696–99.

29 Ducharme J, Farinotti R. Clinical pharmacokinetics and metabolism of chloroquine: focus on recent advancements. *Clin Pharmacokinet* 1996; **31:** 257–74.