Intermittent preventive treatment with dihydroartemisinin–piperaquine and risk of malaria following cessation in young Ugandan children: a double-blind, randomised, controlled trial

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Summary

Background Intermittent preventive treatment (IPT) of malaria with dihydroartemisinin–piperaquine is a promising strategy for malaria prevention in young African children. However, the optimal dosing strategy is unclear and conflicting evidence exists regarding the risk of malaria after cessation of chemoprevention. We aimed to compare two dosing strategies of IPT with dihydroartemisinin–piperaquine in young Ugandan children, and to evaluate the risk of malaria after cessation of IPT.

Methods In this double-blind, randomised controlled phase 2 trial, women and their unborn children were recruited at Tororo District Hospital (Tororo, Uganda). Eligible participants were HIV-negative women aged 16 years or older with a viable pregnancy (gestational age 12–20 weeks). Women and their unborn children were randomly assigned (1:1:1:1) to one of four treatment groups, all receiving dihydroartemisinin–piperaquine, on the basis of the IPT intervention received by the woman during pregnancy: women every 8 weeks, children every 4 weeks; women every 4 weeks, children every 4 weeks; women every 8 weeks, children every 12 weeks; and women every 4 weeks, children every 12 weeks. Block randomisation was done by an independent investigator using a computer-generated randomisation list (permuted block sizes of six and 12). We analysed children on the basis of their random assignment to receive dihydroartemisinin–piperaquine (20 mg/160 mg tablets) once daily for 3 consecutive days every 4 weeks or 12 weeks. Children received study drugs from age 8 weeks to 24 months and were followed-up to age 36 months. Participants and investigators were masked to treatment allocation. The primary outcome was the incidence of symptomatic malaria during the intervention and following cessation of the intervention, adjusted for potential confounders. The primary outcome and safety were assessed in the modified intention-to-treat population, which included all children who reached 8 weeks of age and received at least one dose of study drug. This trial is registered with ClinicalTrials.gov, number NCT02163447.

Findings Between Oct 21, 2014, and May 18, 2015, 191 children were born, of whom 183 reached 8 weeks of age and received at least one dose of study drug and thus were included in the primary analysis (96 children in the 4-week group and 87 in the 12-week group). During the intervention, the incidence of symptomatic malaria was significantly lower among children treated every 4 weeks than children treated every 12 weeks; three episodes occurred among children treated every 4 weeks (incidence 0·018 episodes per person-year) compared with 61 episodes among children treated every 12 weeks (incidence 0·39 episodes per person-year; adjusted incidence rate ratio [aIRR] 0·041, 95% CI 0·012–0·150, p<0·0001). After cessation of IPT, children who had previously received dihydroartemisinin–piperaquine every 4 weeks had a lower incidence of symptomatic malaria than children who were treated every 12 weeks; 62 episodes occurred among children previously treated every 4 weeks (incidence 0·73 episodes per person-year) compared with 83 episodes among children treated every 12 weeks (incidence 1·1 episodes per person-year; aIRR 0·62, 0·40–0·95, p=0·028). In the 4-week group, 94 (98%) of 96 children had adverse events versus 87 (100%) of 87 children in the 12-week group. The most commonly reported adverse event was cough in both treatment groups (94 [98%] in the 4-week group *vs* 87 [100%] in the 12-week group). 16 children had severe adverse events (seven [7%] children in the 4-week group *vs* nine [10%] children in the 12-week group). No severe adverse events were thought to be related to study drug administration. One death occurred during the intervention (age 8 weeks to 24 months), which was due to respiratory failure unrelated to malaria.

Interpretation IPT with dihydroartemisinin–piperaquine given every 4 weeks was superior to treatment every 12 weeks for the prevention of malaria during childhood, and this protection was extended for up to 1 year after cessation of IPT.

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Research in context

Evidence before this study

Intermittent preventative treatment (IPT) with antimalarial drugs is a strategy for the control of malaria in African children, but little evidence is available regarding appropriate drugs to use in areas with perennial transmission or high prevalence of sulfadoxine–pyrimethamine resistance. Dihydroartemisinin–piperaquine is an attractive choice for IPT in these settings because of its safety, efficacy, and long half-life. We searched PubMed for original articles published in English between Jan 1, 2000, and June 1, 2018, using the search term "dihydroartemisinin-piperaquine AND prevention AND childhood". Our search yielded five randomised controlled trials of dihydroartemisinin–piperaquine done in Africa for the prevention of malaria in children aged younger than 5 years. In three trials assessing the use of monthly dihydroartemisinin–piperaquine as seasonal malaria chemoprevention in the setting of low sulfadoxine–pyrimethamine resistance, dihydroartemisinin–piperaquine was found to be similarly efficacious to regimens containing either sulfadoxine–pyrimethamine plus amodiaquine or sulfadoxine–pyrimethamine plus piperaquine. Two other trials done in a setting of perennial transmission and high sulfadoxine–pyrimethamine resistance compared the use of monthly dihydroartemisinin–piperaquine as IPT with monthly sulfadoxine–pyremethamine, daily trimethoprim–sulfamethoxazole (co-trimoxazole), or no chemoprevention among children aged between 6 months and 2 years. In these trials, dihydroartemisinin–piperaquine was found to be significantly more efficacious than sulfadoxine–pyrimethamine or no chemoprevention, but efficacy seemed to be limited by inadequate drug exposure or poor adherence. These two trials also assessed the incidence of malaria after cessation of the study drug and found no evidence of so-called rebound malaria among children previously given dihydroartemisinin–piperaquine. In all trials, dihydroartemisinin–piperaquine was found to be as safe and well tolerated.

Added value of this study

To our knowledge, this is the first study to compare different dosing strategies of IPT with dihydroartemisinin–piperaquine for the prevention of malaria in young children. This is also the first trial to compare the risk of malaria following cessation of IPT among children previously given directly observed IPT. In a setting where malaria transmission had been reduced substantially due to indoor residual spraying of insecticides, IPT with dihydroartemisinin–piperaquine given every 4 weeks was superior to IPT with dihydroartemisinin–piperaquine given every 12 weeks for the prevention of malaria between age 8 weeks and 2 years. Furthermore, this protection was sustained for up to 1 year after cessation of IPT. Both regimens were well tolerated and safe.

Implications of all the available evidence

IPT with dihydroartemisinin–piperaquine every 4 weeks almost eliminated malaria in young children and was associated with less malaria 1 year after cessation than IPT with dihydroartemisinin–piperaquine given every 12 weeks. Dihydroartemisinin–piperaquine should be strongly considered for IPT in settings where transmission occurs throughout the year and resistance to sulfadoxine–pyrimethamine is high.

Introduction

Controlling, and ultimately eliminating malaria in high transmission settings such as Uganda remains a major challenge. Partial immunity to malaria develops through repeated exposure, leading first to protection against severe forms of disease, followed by protection against symptomatic illness.[1] Therefore, in highly endemic areas, the burden of malaria is heavily borne by young children (aged >5 years). The only widely used tool for the prevention of malaria in African children is long-lasting insecticidal nets (LLINs). However, concerns have been raised about the efficacy of LLINs, which might be diminishing due to the emergence of vector resistance to pyrethroids.[2] WHO also recommends indoor residual spraying of insecticides (IRS) as a central part of malaria control policy because of its efficacy in reducing vector densities and malaria morbidity,[3,4] but coverage rates have been low (<10%), possibly due to costs from spraying with non-pyrethroid insecticides.[5] Optimisation of currently deployed interventions and the development of innovative strategies to prevent malaria in early childhood are urgently needed.

The use of intermittent preventative treatment (IPT) with antimalarial drugs for African children at high risk for malaria is an effective option in certain settings. In areas with a low prevalence of sulfadoxine–pyrimethamine resistance, IPT with sulfadoxine–pyrimethamine given at the time of routine vaccination in infants has been shown to be safe and effective against malaria in the first year of life.[6] In settings where malaria transmission is highly seasonal, seasonal malaria chemoprevention with sulfadoxine–pyrimethamine plus amodiaquine has been found to be highly effective and safe.[7] However, IPT in infants and seasonal malaria chemoprevention are not recommended in areas with high prevalence of sulfadoxine–pyrimethamine resistance or perennial malaria transmission (eg, much of central and east Africa).[5] In these settings, the safe, highly efficacious artemisinin-based combination therapy dihydroartemisinin–piperaquine has emerged as an excellent candidate for use as IPT in children because of its prolonged post-treatment prophylaxis.[8–12] In two studies done in the same high transmission setting, IPT with dihydroartemisinin–piperaquine given monthly was associated with a 58% protective efficacy among

HIV-unexposed children,[11] and 69% protective efficacy among HIV-exposed uninfected children,[12] compared with no chemoprevention. However, these studies were open-label, and study drugs were not directly observed, leading to high levels of inadequate drug exposure and probable non-adherence. Among a subset of children with high piperaquine exposure, dihydroartemisinin–piperaquine was nearly 100% protective against malaria.[13]

Although IPT might be effective in preventing malaria in children, a potential concern is the effect chemoprevention could have on the acquisition of anti-malarial immunity and risk of malaria after chemoprevention has stopped. Some studies[14,15] have reported that children receiving chemoprevention have an increased risk of symptomatic malaria following cessation compared with children who do not receive chemoprevention (ie, so-called rebound malaria), whereas other studies[16] have reported either no rebound[6] or evidence of sustained protection following cessation. In our previous study[11] of IPT with dihydroartemisinin–piperaquine given to children between age 6 months and 24 months done in a high transmission setting in Uganda, no difference in the per-protocol risk of symptomatic malaria was observed between children who had previously received the study drug every 4 weeks and those who had not received chemoprevention in the year after cessation. However, in a post-hoc analysis in which piperaquine exposure during the intervention was considered a marker of drug adherence, children highly adherent to the study drug had a 97% reduction in symptomatic malaria during the time the intervention was given[13] and a 55% reduction in symptomatic malaria in the year following cessation compared with children given sulfadoxine–pyrimethamine.[17] This difference might be a result of improved priming of cellular immune responses,[17] which has been observed in several animal and experimental models[18–20] whereby parasitaemia is suppressed with drugs that are active only against erythrocytic stages.

See Online for appendix

Although IPT with dihydroartemisinin–piperaquine is effective at preventing malaria in young children, it is unclear whether the potential greater efficacy of more frequent dosing will outweigh the practical benefits of less frequent dosing. Furthermore, conflicting evidence exists with regard to the risk of malaria following cessation of chemoprevention. This study aimed to compare the efficacy of IPT with dihydroartemisinin–piperaquine given either every 4 weeks or every 12 weeks between the age of 8 weeks and 24 months in Ugandan children, and to compare the risk of malaria in these groups after cessation of the study drug.

Methods

Study design and participants

In this double-blind, randomised, controlled phase 2 trial, participants were recruited at Tororo District Hospital (Tororo, Uganda), in an area with a high prevalence of perennial transmission of malaria. Following a LLINs campaign in November, 2013, near universal LLIN coverage was reported in the Tororo district.[4] Between December, 2014, and February, 2015, IRS using the carbamate bendiocarb was initiated in Tororo;[4] subsequent rounds of IRS using bendiocarb were done between June, 2015, and July 2015, and November 2015, and December, 2015. A fourth phase of IRS was completed between June, 2016, and July 2016, using pyrimiphos-methyl (Actellic), a long-lasting organophosphate, and a fifth phase of IRS using Actellic was completed between June and July, 2017.

In the first phase of this study, eligible participants were HIV-negative women aged 16 years or older with a viable pregnancy (estimated gestational age 12–20 weeks) confirmed by a positive urine pregnancy test or ultrasound. Participants were required to provide written informed consent, agree to come to the study clinic for any illness, to avoid medications given outside the study protocol, and planned to deliver in the hospital. Women with chronic medical problems or active medical problems requiring inpatient evaluation were excluded. Full inclusion and exclusion criteria are provided in the study protocol (appendix p 22).[21] In the second phase of the study, all children born to these mothers were enrolled.

The study was approved by the ethics committees of Makerere University School of Biomedical Sciences, the Uganda National Council for Science and Technology, and the University of California, San Francisco. Written informed consent was provided by all study participants.

Randomisation and masking

Women and their unborn children were randomly assigned (1:1:1:1) to one of the following four treatment groups, all receiving dihydroartemisinin–piperaquine, on the basis of the IPT intervention received by the woman during pregnancy: women every 8 weeks, children every 4 weeks; women every 4 weeks, children every 4 weeks; women every 8 weeks, children every 12 weeks; and women every 4 weeks, children every 12 weeks. We analysed children on the basis of their random assignment to receive the study drug every 4 weeks or every 12 weeks. Children were assigned to a treatment group at the time their mothers were randomly assigned in phase 1 of the study with concealment by use of opaque, consecutively numbered, sealed envelopes. Block randomisation was done by an independent investigator using a computer-generated randomisation list (block sizes of six and 12). Pharmacists who were not otherwise involved in the study were responsible for treatment allocation and preparation of study drugs. Placebos were given every 4 weeks to ensure that all participants received the same number of pills. The active drug and the placebo were identical in appearance. All study doses were dispensed by a study nurse from whom treatment allocation was masked.

Procedures

Children had a standardised examination at birth and received standard neonatal care including immunisation, ophthalmic tetracycline, and vitamin K and A supplementation. Children received all medical care at a dedicated study clinic.

Between age 8 weeks and 24 months, children were given half strength dihydroartemisinin–piperaquine tablets (20 mg/160 mg tablets [Duo-Cotecxin, Holley-Cotec, Beijing, China]) once daily for 3 consecutive days according to weight-based guidelines (appendix p 68) every 4 weeks or every 12 weeks. Children randomly assigned to dihydroartemisinin–piperaquine every 12 weeks received a placebo every 4 weeks when they were not receiving active study drugs. Administration of the first daily doses were directly observed in the clinic. The second and third daily doses were administered by the child's parent or guardian at home using pre-packaged drugs in opaque envelopes. Parents or guardians were instructed to bring the child to the study clinic in case of vomiting within 30 min of drug administration or if study drug was lost.

Routine visits were done every 4 weeks, including assessment of adherence to study drugs administered at home and LLIN use, and collection of blood for the detection of parasites. Routine laboratory testing for complete blood counts and alanine aminotransferase concentrations was done every 16 weeks. Adverse events were assessed and graded according to standardised criteria[22] at every visit to the study clinic. Mothers were encouraged to bring their children to the study clinic any time their children were ill. Children who presented with documented fever (tympanic temperature >38·0°C) or history of fever in the previous 24 h had blood collected for a thick blood smear for the detection of malaria parasites. If the smear was positive, the child was diagnosed with symptomatic malaria and treated with artemether–lumefantrine for uncomplicated cases. Episodes of complicated malaria or treatment failures occurring within 14 days of previous treatment were treated with artesunate or quinine according to 2016 Uganda Clinical Treatment Guidelines.[23]

Blood smears were stained with 2% Giemsa and read by experienced laboratory technicians. A blood smear was considered negative when the examination of 100 high-power fields revealed no asexual parasites. For quality control, all slides were read by a second microscopist, and a third reader settled any discrepancies. Dried blood spots were collected from study participants every 4 weeks and tested for the presence of malaria parasites using a loop-mediated isothermal amplification (LAMP) kit (Eiken Chemical, Tokyo, Japan).

Children were followed up until age 36 months. Study participants were withdrawn from the study if they were unable to be located for more than 60 days, moved out of the study area, died, withdrew informed consent, or did not comply with study activities.

Outcomes

The primary outcome was the incidence of symptomatic malaria among children, defined as the number of incident episodes per time at risk. An incident episode was defined as a symptomatic malaria episode requiring treatment and not preceded by another episode in the previous 14 days. For comparison of symptomatic malaria incidence during the intervention, time at risk was defined as the time from birth to age 24 months, or early study termination (if before age 24 months). For the comparison of the incidence of symptomatic malaria

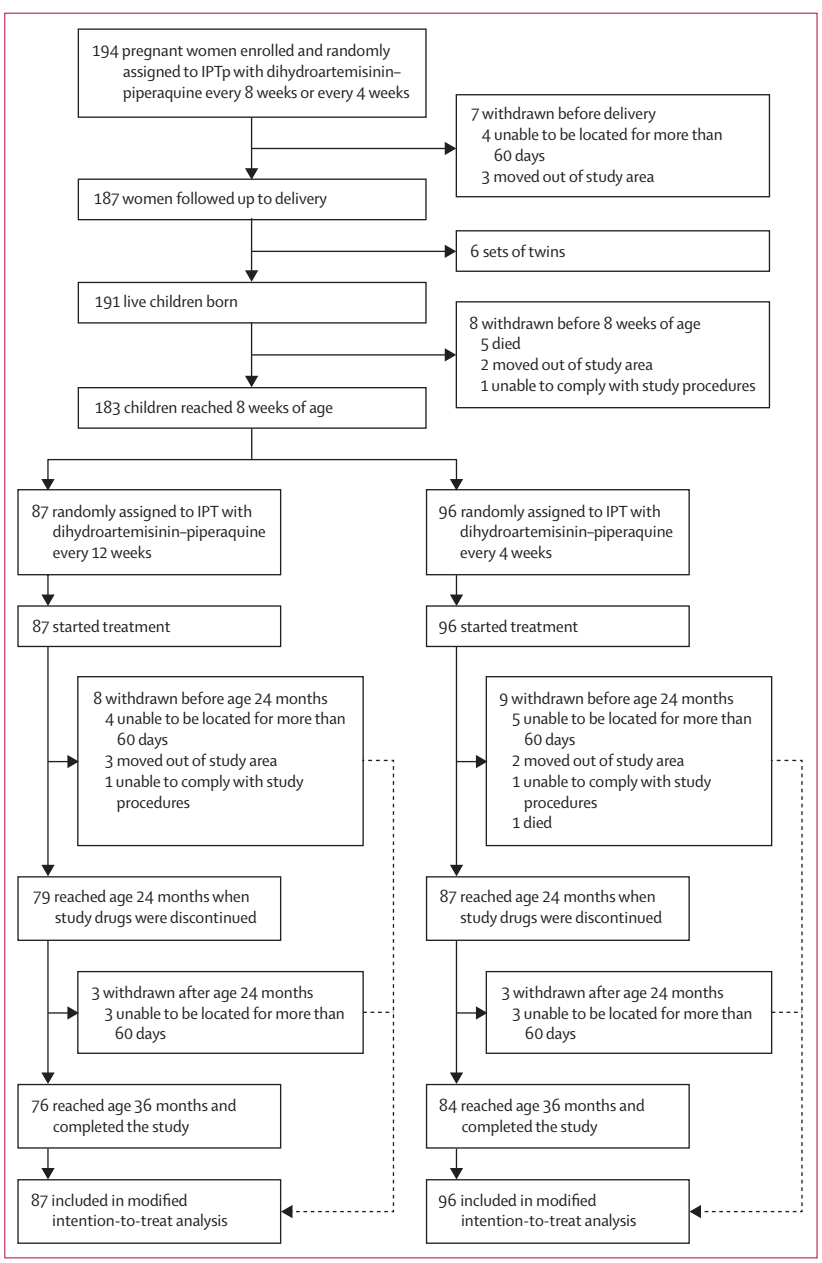

Figure 1: **Trial profile**
IPTp=intermittent preventive treatment in pregnancy. IPT=intermittent preventive treatment.

following cessation of study drugs, the follow-up period between age 24 and 36 months was considered the time at risk. Secondary outcomes were incidence of complicated malaria; incidence of hospital admissions and deaths; incidence of non-malarial febrile illness (presentation within 14 days of a previous episode were not considered incident events); the prevalence of parasitaemia (microscopic and submicroscopic parasitaemia assessed by LAMP) measured at routine monthly visits; prevalence of anaemia (proportion of haemoglobin measurements <11 g/dL and <8 g/dL) measured at routine visits every 16 weeks; parasite densities if parasitaemic; and immunological measurements (antibody and cellular responses measured at birth, and age 6, 12, and 24 months). Measures of safety and tolerability included observed and reported vomiting after the administration of study drugs and the incidence of grade 3 and 4 adverse events from 8 weeks of age to 1 month after administration of the last dose of study drugs. Immunological assays are ongoing, and thus results for this secondary outcome have not been reported.

	Dihydroartemisinin–piperaquine every 12 weeks (n=87)	Dihydroartemisinin–piperaquine every 4 weeks (n=96)
Characteristics of mothers at enrolment		
Age, years	23·1 (4·0)	22·0 (4·4)
Gravidity		
1	20 (23%)	43 (45%)
2	30 (35%)	24 (25%)
≥3	37 (43%)	29 (30%)
Household wealth index*		
Lowest third tertile	25 (29%)	35 (37%)
Middle third tertile	32 (37%)	30 (31%)
Highest third tertile	30 (35%)	31 (32%)
Detection of malaria parasites by LAMP†	43 (49%)	60 (63%)
Maternal IPTp regimen		
Dihydroartemisinin–piperaquine every 8 weeks	43 (49%)	46 (48%)
Dihydroartemisinin–piperaquine every 4 weeks	44 (51%)	50 (52%)
Characteristics of children at birth		
Gestational age at birth, weeks	40 (32–42)	39 (30–43)
Sex		
Girls	44 (51%)	45 (47%)
Boys	43 (49%)	51 (53%)
Birth weight, g	2981 (1320–3840)	2862 (1240–3800)
Placental malaria by microscopy	1/86 (1%)	2/95 (2%)
Placental malaria by LAMP	1/86 (1%)	6/95 (6%)
Placental malaria by histology	23/86 (27%)	34/95 (36%)

Data are mean (SD), n (%), mean (range), or n/N (%). IPT=intermittent preventive treatment. LAMP=loop amplified isothermal amplification. IPTp=intermittent preventive treatment in pregnancy. *Wealth was measured by the reported ownership of household items such as telephones, radios, and bicycles, which was then aggregated in a principal component analysis and categorised into lowest, middle, and highest tertiles of household wealth index.[24] †Proportion of routine blood samples positive for malaria parasites by LAMP.

Table 1: **Baseline characteristics of mothers and children by treatment group**

Statistical analysis

Our target sample size was determined on the basis of the hypothesis that children randomly assigned to receive dihydroartemisinin–piperaquine every 4 weeks would have a lower incidence of symptomatic malaria following cessation of chemoprevention than children given dihydroartemisinin–piperaquine every 12 weeks, since the magnitude of differences for this outcome was expected to be smaller than those anticipated for other outcomes (appendix p 42). During the chemoprevention intervention (age 8 weeks to 24 months), we assumed an incidence of symptomatic malaria of three to five episodes per person-year among children given dihydroartemisinin–piperaquine every 12 weeks based on previous data[11] obtained before the implementation of IRS; assuming 5% of children were lost to follow-up per year, 180 participants would be needed to detect a 18–23% reduction among children randomly assigned to receive dihydroartemisinin–piperaquine every 4 weeks with 80% power and a two-sided significance level of 5%. Following cessation of chemoprevention (age 24–36 months), we assumed an incidence of symptomatic malaria of three to five episodes per person-year among children randomly assigned to receive dihydroartemisinin–piperaquine every 12 weeks; assu-ming 5% of children were lost to follow-up per year, 164 participants would be needed to detect a 16–21% reduction among children randomised to receive dihydroartemisinin–piperaquine every 4 weeks with 80% power and a two-sided significance level of 5%.

Data were double-entered and verified in Microsoft Access (version 14.0), and statistical analyses were done using Stata (version 15). All analyses were done in the modified intention-to-treat population, which included all children who reached 8 weeks of age and received at least one dose of study drug. Incident outcomes were compared using negative binomial regression. Prevalence measures were compared using generalised estimating equations with robust SEs to account for repeated measures within participants. The cumulative risk of developing symptomatic malaria from cessation of study drugs was estimated using the Kaplan–Meier product limit formula, and associations with IPT regimens were assessed using a Cox proportional hazards model. The cumulative risk of developing repeated symptomatic malaria following cessation of study drugs was estimated using a within-subject variance-corrected Cox proportional hazards model. In all analyses, estimates were adjusted for covariates found to be imbalanced between treatment groups; both unadjusted and adjusted results were reported in tables and adjusted estimates are presented in the text and in figures. In all analyses, a two-sided p value of less than 0·05 was considered to indicate a statistically significant difference. This trial is registered with ClinicalTrials.gov (NCT02163447).

	Dihydroartemisinin–piperaquine every 12 weeks* (n=87)	Dihydroartemisinin–piperaquine every 4 weeks (n=96)	Unadjusted IRR or prevalence RR (95% CI)	p value	Adjusted IRR or prevalence RR (95% CI)†	p value
Incidence of symptomatic malaria	61 (0·39)	3 (0·018)	0·045 (0·013–0·16)	<0·0001	0·041 (0·012–0·15)	<0·00001
Microscopic parasitaemia‡	69/2021 (3%)	3/2186 (<1%)	0·039 (0·012–0·13)	<0·0001	0·037 (0·01–0·12)	<0·0001
Microscopic or submicroscopic parasitaemia‡	99/2010 (5%)	13/2173 (1%)	0·12 (0·053–0·28)	<0·0001	0·11 (0·052–0·25)	<0·0001
Anaemia§	222/486 (46%)	247/518 (48%)	1·0 (0·87–1·2)	0·65	1·0 (0·86–1·2)	0·79
Incidence of non-malarial febrile illnesses	555 (3·6)	599 (3·6)	1·0 (0·84–1·2)	0·98	1·0 (0·83–1·2)	0·97
Incidence of hospitalisation or death	5 (0·032)	7 (0·042)	1·3 (0·39–4·5)	0·65	2·0 (0·58–6·7)	0·27

Data are episodes (episodes per person-year at risk) or n/N (%). IRR=incidence rate ratio. RR=rate ratio. *Reference group. †Adjusted for maternal gravidity and maternal parasitaemia at enrolment. ‡Measured at routine visits done every 4 weeks, including any episode of malaria diagnosed within the previous 28 days. §Haemoglobin concentration of less than 11 g/dL measured at routine visits done every 16 weeks.

Table 2: **Protective efficacy of intermittent preventive treatment with dihydroartemisinin–piperaquine given every 12 weeks versus every 4 weeks between age 8 weeks and 24 months**

Role of the funding source

The funder 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 in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 21, 2014, and May 15, 2015, 191 children were born to 194 mothers enrolled and followed until delivery (figure 1). Of these 191 children, 183 children reached 8 weeks of age, and received at least one dose of study drug and thus were included in the analysis (96 children in the 4 week dihydroartemisinin–piperaquine group and 87 children in the 12 week dihydroartemisinin–piperaquine group). Mothers of children randomly assigned to receive the study drug every 4 weeks were significantly more likely to be primigravid than mothers of children randomly assigned to receive treatment every 12 weeks (45% *vs* 23%, p=0·0019) and more likely to have parasitaemia at enrolment, but this difference was non-significant (63% *vs* 49%, p=0·075; table 1). Other baseline characteristics of mothers and children were similar between groups (table 1).

Among the 183 children analysed, 166 (91%) were followed-up to age 24 months and 106 (87%) to 36 months. The mean duration of follow-up was 32 months (SD 6·4), and this was not significantly different between treatment groups (p=0·45). At 6263 (99%) of 6316 monthly assessments, mothers and children reported sleeping under an LLIN the previous evening, with no significant differences between the treatment groups (p=0·27). During the chemo-prevention phase, as a result of premature study withdrawal, 57 (3%) of 2175 potential visits were missed by children given study drug every 12 weeks and 112 (5%) of 2400 potential visits were missed by children given study drug every 4 weeks. Among children who were actively followed up, study drugs were not given at nine (<1%) of 2118 scheduled visits among children randomly assigned to receive the study drug every 12 weeks and at eight (<1%) of 2288 scheduled visits among children randomly assigned to receive the study drug every 4 weeks.

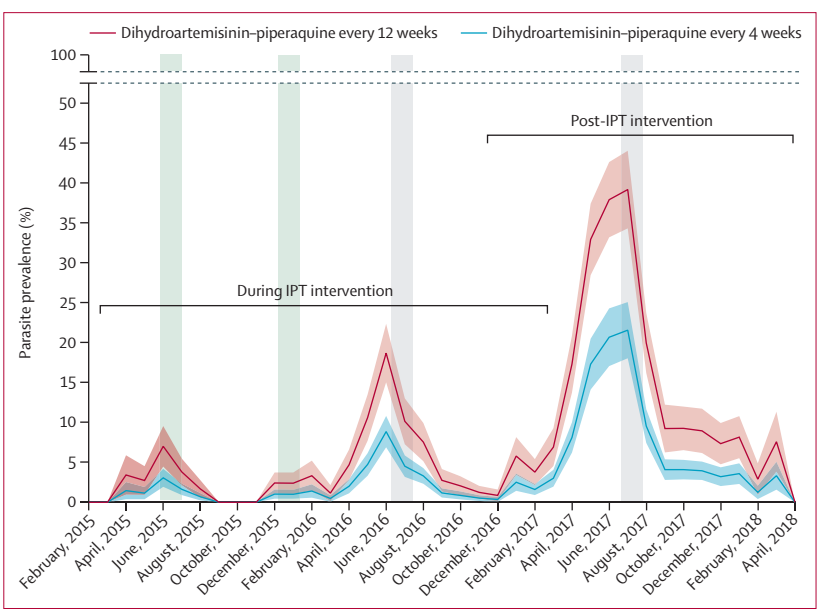

Figure 2: **Effect of IPT with dihydroartemisinin–piperaquine on parasite prevalence during treatment and after cessation of intervention**

Parasite prevalence was assessed monthly by microscopy and loop mediated isothermal amplification. Vertical bars represent phases of indoor residual spraying of insecticides (green is spraying with bendiocarb, grey is spraying with pirimiphos-methyl). IPT=intermittent preventive treatment.

No episodes of symptomatic malaria were identified before the initiation of treatment with the study drug. During the chemoprevention intervention phase (age 8 weeks to 24 months), 64 episodes of symptomatic malaria were identified (incidence 0·20 episodes per person-year); three episodes among children who received study drug every 4 weeks (incidence 0·018 episodes per person-year) and 61 episodes among children who received study drug every 12 weeks (incidence 0·39 episodes per person-year, table 2). The incidence of symptomatic malaria was 96% lower among children who received treatment every 4 weeks than children who received treatment every 12 weeks (adjusted incidence rate ratio [aIRR] 0·04, 95% CI 0·012–0·150, p<0·0001). Three (3%) of 96 children who received the study drug every 4 weeks developed symptomatic malaria compared with 29 (33%) of 87 children who received study drug every 12 weeks. The

	Dihydroartemisinin–piperaquine every 12 weeks (n=87)	Dihydroartemisinin–piperaquine every 4 weeks (n=96)
Vomiting following administration of study drugs*		
Observed after administration of first dose in clinic	33/2109 (2%)	99/2280 (4%)†
Reported after administration of second dose at home	40/2102 (2%)	65/2270 (3%)
Reported after administration of third dose at home	18/2102 (1%)	34/2270 (1%)
Adverse events of any severity‡		
Cough	998 (6·2)	1127 (6·4)
Diarrhoea	415 (2·6)	470 (2·7)
Vomiting	108 (0·67)	114 (0·65)
Rash	35 (0·22)	43 (0·25)
Conjunctivitis	25 (0·15)	34 (0·19)
Anorexia	24 (0·15)	18 (0·10)
Malaise	10 (0·064)	10 (0·059)
Individual grade 3–4 adverse events		
Anaemia	2 (0·012)	4 (0·023)
Thrombocytopenia	2 (0·012)	4 (0·023)
Respiratory distress	1 (0·0064)	1 (0·0059)
Elevated alanine aminotransferase	0 (0)	2 (0·011)
Cough	0 (0)	2 (0·011)
Diarrhoea	1 (0·0064)	1 (0·0059)
Seizures	0 (0)	1 (0·0059)
Neutropenia	0 (0)	1 (0·0059)
Altered mental status	1 (0·0064)	0 (0)
Dehydration	1 (0·0064)	0 (0)
Malnutrition	0 (0)	1 (0·0059)
All grade 3–4 adverse events	8 (0·049)	17 (0·097)
Grade 3–4 adverse events possibly related to study drugs	0 (0)	0 (0)
All serious adverse events	7 (0·043)	12 (0·069)

Data are n/N (%) or episodes (episodes per person-year at risk). *Including both active study drug and placebo. †p=0·001. ‡Adverse events for which at least ten total events were reported.

Table 3: **Safety outcomes**

prevalence of parasitaemia detected by microscopy or LAMP was 89% lower among children who received study drug every 4 weeks than children who received study drug every 12 weeks (adjusted prevalence RR 0·11, 95% CI 0·052–0·25, p<0·001; figure 2). Five episodes of complicated malaria were identified, all of which occurred among children given dihydroartemisinin–piperaquine every 12 weeks. The prevalence of anaemia and the incidence of non-malarial febrile illnesses were similar between the treatment groups (table 2). During the intervention, 11 hospital admissions and one death were reported, with no significant differences observed between the study groups (table 2). The death was due to respiratory failure unrelated to malaria and occurred in a 5-month-old child randomly assigned to receive dihydroartemisinin–piperaquine every 4 weeks.

Overall, the prevalence and incidence of adverse events were low and similar between the two treatment groups (table 3); 94 (98%) of 96 children in the 4-week group and 87 (100%) of 87 children in the 12-week group had adverse events. The most commonly reported adverse event was cough in both treatment groups (94 [98%] in the 4-week group *vs* 87 [100%] in the 12-week group). Vomiting after the first dose of drug administration was more common in children receiving study drug every 4 weeks than children given study drug every 12 weeks (4·3% *vs* 1·6%, p=0·0010). Vomiting after the second and third administration of the study drugs occurred in less than 3% of children with no significant difference between groups. 25 grade 3–4 adverse events and 19 serious adverse events were reported; the incidence of adverse events was similar between the two study groups. No grade 3–4 treatment-related adverse events or serious adverse events were reported.

Between age 24–36 months, after cessation of study drugs, 145 episodes of symptomatic malaria were reported; 62 among children previously given the study drug every 4 weeks (incidence 0·73 episodes per person-year) and 83 among children previously given the study drug every 12 weeks (incidence 1·1 episodes per person-year; table 4). The incidence of symptomatic malaria was 38% lower among children who had previously received the study drug every 4 weeks than children who had received the study drug every 12 weeks (aIRR 0·62, 95% CI 0·40–0·95, p=0·028). Similarly, the prevalence of parasitaemia detected by microscopy or LAMP was 41% lower after cessation of study drugs among children who had previously received treatment every 4 weeks than children who had received treatment every 12 weeks (aIRR 0·59 0·40–0·86, p=0·0061; table 4). The prevalence of parasitaemia was highest before each round of IRS, although the protective efficacy of dihydroartemisinin–piperaquine every 4 weeks was similar both before and after IRS (figure 2). The risk of a first symptomatic malaria episode following cessation of study drugs at age 24 months was lower among children who had previously received the study drug every 4 weeks than children who had received the study drug every 12 weeks, but this difference was not statistically significant (adjusted hazard ratio [aHR] 0·72, 95% CI 0·45–1·1, p=0·17; figure 3A). However, the risk of repeated symptomatic malaria following either cessation of study drugs or malaria treatment was significantly lower among children who had previously received treatment every 4 weeks than children who had received treatment every 12 weeks (aHR 0·68, 95% CI 0·48–0·94, p=0·021; figure 3B). Seven episodes of complicated malaria were reported; four among children who had previously received the study drug every 4 weeks and three among children who had previously received the study drug every 12 weeks. The prevalence of anaemia and incidence of non-malaria febrile illnesses were similar between the treatment groups (table 4). Overall, nine hospital admissions were reported between drug cessation at

	Dihydroartemisinin–piperaquine every 12 weeks* (n=79)	Dihydroartemisinin–piperaquine every 4 weeks (n=87)	Unadjusted IRR or prevalence RR (95% CI)	p value	Adjusted IRR or prevalence RR (95% CI)†	p value
Incidence of symptomatic malaria	83 (1·1)	62 (0·73)	0·70 (0·46–1·1)	0·088	0·62 (0·40–0·95)	0·028
Microscopic parasitaemia‡	109/1011 (11%)	77/1089 (7%)	0·66 (0·46–0·96)	0·030	0·60 (0·41–0·88)	0·0082
Microscopic or submicroscopic parasitaemia‡	148/996 (15%)	103/1077 (10%)	0·65 (0·45–0·95)	0·025	0·59 (0·40–0·86)	0·0061
Anaemia§	56/217 (26%)	51/236 (22%)	0·83 (0·55–1·3)	0·38	0·83 (0·55–1·2)	0·36
Incidence of non-malarial febrile illnesses	216 (2·75)	235 (2·77)	1·0 (0·82–1·2)	0·93	1·0 (0·81–1·2)	1·0
Incidence of hospitalisation or death	2 (0·025)	7 (0·083)	3·2 (0·67–16)	0·14	4·2 (0·83–21)	0·082

Data are episodes (episodes per person-year at risk) or n/N (%). IRR=incidence rate ratio. RR=rate ratio. *Reference group. †Adjusted for maternal gravidity and maternal parasitaemia at enrolment. ‡Measured at routine visits every 4 weeks, including any episode of malaria diagnosed in the previous 28 days. §Haemoglobin concentration less than 11 g/dL at routine visits done every 16 weeks.

Table 4: **Malaria and non-malarial outcomes between age 24–36 months after cessation of intermittent preventive treatment**

24 months and age 36 months; seven among children who received the study drug every 4 weeks and two among children who received the study drug every 12 weeks, but these differences were not statistically significant (table 4). No deaths occurred after cessation of IPT.

Discussion

Our findings showed that children given IPT with dihydroartemisinin–piperaquine every 4 weeks had 96% fewer episodes of symptomatic malaria than children who received dihydroartemisinin–piperaquine every 12 weeks. Additionally, the prevalence of parasitaemia was 89% lower among children in the 4-week treatment group than those in the 12-week treatment group. Both regimens were safe and well tolerated. Importantly, after discontinuation of study drugs, children who had previously received IPT with the study drug every 4 weeks had 39% fewer episodes of symptomatic malaria and 42% lower prevalence of parasitaemia than children who had previously received the study drug every 12 weeks. Together, these data suggest that IPT with dihydroartemisinin–piperaquine given every 4 weeks in young children is highly protective against malaria, and, rather than resulting in so-called rebound malaria after stopping IPT, children continued to have sustained protection for up to 1 year.

IPT with anti-malarial drugs for infants and children at high risk for malaria has been shown to be safe and effective for prevention of malaria; however, several issues have prevented its wide-scale deployment. Although IPT with sulfadoxine–pyrimethamine was protective against symptomatic malaria in infants in the early 2000s,[6] since then, anti-folate resistance has become widespread, especially in east Africa,[25] limiting its implementation. Similarly, although seasonal malaria chemoprevention has been shown to have an overall protective efficacy of 82% against symptomatic malaria in areas of west Africa with highly seasonal malaria,[7] seasonal malaria chemoprevention would not be appropriate in most areas of central and east Africa where transmission is perennial. The high efficacy, safety, and prolonged post-treatment prophylaxis of dihydroartemisinin–piperaquine makes it an excellent candidate for use as IPT in areas with perennial transmission and widespread sulfadoxine–pyrimethamine resistance. By comparison with sulfadoxine–pyrimethamine,

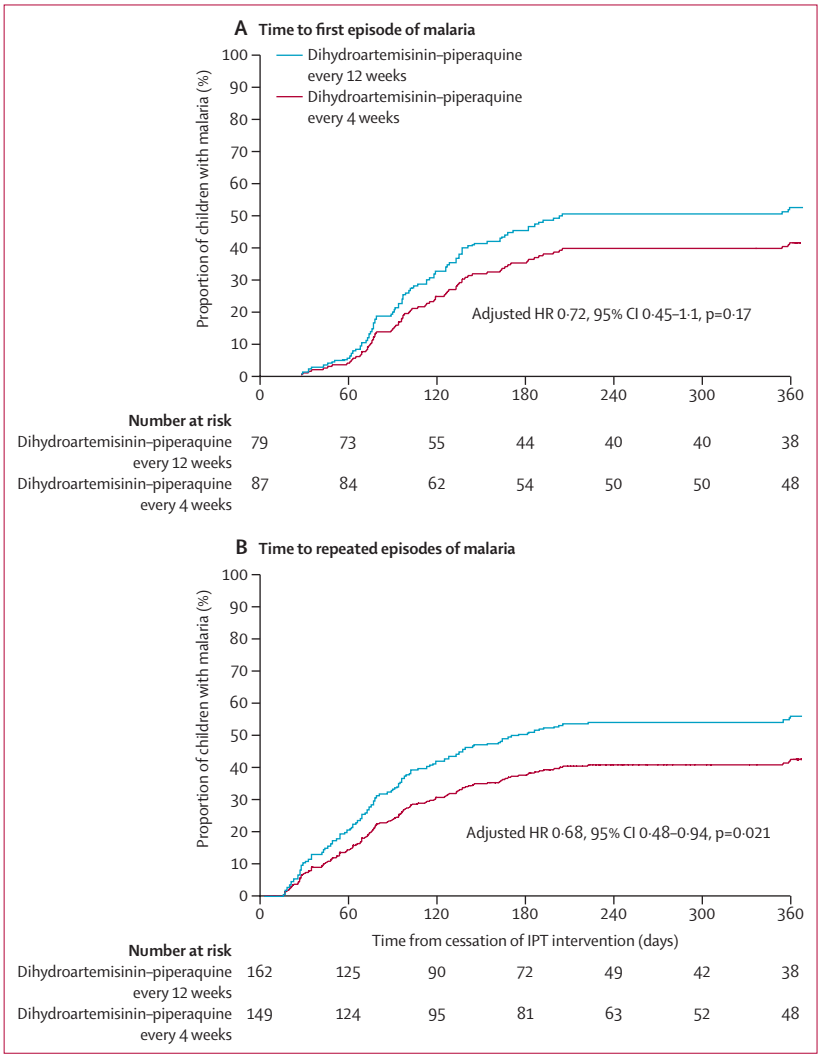

Figure 3: **Time to malaria following cessation of IPT intervention**
Time to first episode (A) or repeated episodes (B) of malaria following cessation of study drugs at age 24 months, adjusted for maternal gravidity and parasitaemia at enrolment. IPT=intermittent preventive treatment. HR=hazard ratio.

dihydroartemisinin–piperaquine has been shown to have more than 90% efficacy against symptomatic malaria and parasitaemia when given as IPT during pregnancy[21] and in school children.[26] In the present study, when dihydroartemisinin–piperaquine was given as directly observed therapy to young children between the ages of 8 weeks and 24 months in the setting of IRS, the incidence of symptomatic malaria was reduced by 96%.

Both dihydroartemisinin–piperaquine regimens were safe and well tolerated overall, and no grade 3 or 4 treatment-related adverse events were observed. The risk of vomiting following administration of the first dose of study drug was higher in children who received IPT with dihydroartemisinin–piperaquine every 4 weeks than in children who received dihydroartemisinin–piperaquine every 12 weeks, which is likely due to the higher number of study drug doses received by children in the 4 week treatment group than the 12 week treatment group.[27] No adverse events suggestive of cardiotoxicity were observed for children enrolled in this study, consistent with a meta-analysis[28] demonstrating the cardiac safety of dihydroartemisinin–piperaquine when used for malaria treatment or prevention. The prevalence of anaemia and hospital admissions were also similar in both IPT treatment groups, which is not surprising considering that adverse outcomes due to symptomatic malaria generally occur after multiple repeated episodes.

Another reason that IPT in children has not been widely implemented is because of concerns that decreasing exposure to malaria might delay the development of natural immunity, leading to an increased risk of malaria when the drugs are stopped. Studies from Tanzania[15] and the Gambia[14] reported that children receiving chemoprophylaxis with pyrimethamine plus dapsone had a higher incidence of symptomatic malaria than those receiving placebo in the year following the intervention. By contrast, one study[16] reported a sustained decrease in symptomatic malaria following cessation of IPT with sulfadoxine–pyrimethamine in infants, but this finding was not reproduced in other studies,[6] and we previously reported that so-called highly adherent children given dihydroartemisinin–piperaquine every 4 weeks had evidence of sustained protection in the year following cessation by comparison with children given no chemoprevention.[17] Differences in these findings could be due to varying transmission intensity between studies (eg, sustained protection might occur in areas of high transmission intensity where recurrent, high-level malaria infection interferes with the development of antimalarial immunity[29]). Alternatively, differences might be due to the protective efficacy or mechanism of action of study drugs or dosing strategies (eg, continuous prophylaxis *vs* intermittent therapy that allows for breakthrough parasitaemia). In the present study, children who had previously received dihydroartemisinin–piperaquine every 4 weeks continued to have fewer episodes of symptomatic malaria and parasitaemia after study drug cessation than did children who had received dihydroartemisinin–piperaquine every 12 weeks. Risk of first symptomatic malaria episode following cessation of study drugs was similar between the two treatment groups, although the risk of multiple episodes was significantly lower among children who had previously received the study drug every 4 weeks compared with those given study drug every 12 weeks. This finding suggests that near complete prevention of malaria in young children with dihydroartemisinin–piperaquine might lead to the acquisition of partial, but sustained, protection against subsequent infections.

Although mechanisms for this sustained protection are unclear, one possibility is that selective blockade of blood-stage infection with dihydroartemisinin–piperaquine might result in a more robust immune response against pre-erythrocytic stage antigens, which has been suggested by experimental vaccination models using radiation or chemoattenuated sporozoites.[20,30,31] Alternatively, recurrent blood-stage parasitaemia has been shown to trigger multiple immunoregulatory mechanisms that might interfere with the development of effective anti-malarial immunity. Prevention of blood-stage infection by use of anti-malarial chemoprevention might prevent induction of these regulatory responses;[17] however, the precise mechanisms mediating this protection remain to be determined.

Our study had a few limitations, including the absence of a control group (ie, no chemoprevention), which precluded our ability to make comparisons between our two IPT with dihydroartemisinin–piperaquine regimens and the current standard of care. However, we felt that it would be unethical to withhold IPT with dihydroartemisinin–piperaquine from children given the findings of our previous studies in the same setting. Baseline characteristics were slightly imbalanced between the two treatment groups: children randomly assigned to receive the study drug every 4 weeks were more likely to be born to primigravid mothers with parasitaemia at enrolment than children given the study drug every 12 weeks. However, since these covariates are associated with a higher risk of malaria in young children, this might have resulted an underestimation of the protective efficacy of dihydroartemisinin–piperaquine every 4 weeks. Our findings might not be readily generalisable to other epidemiological settings, such as areas where IRS has not been implemented or among children born to HIV-positive mothers, although we have previously shown that monthly dihydroartemisinin–piperaquine was well tolerated and associated with a significant reduction in symptomatic malaria in young HIV-exposed children compared with no chemoprevention.[12] Only the first dose of study drug was directly observed, and failure to take the two doses administered at home could have differentially affected the outcomes for the treatment groups. However, these missed doses were unlikely to

have been a significant factor, since any bias would likely be towards the null. Additionally, we did not follow-up children after age 3 years, thereby limiting our ability to assess further the effect of chemoprevention cessation on symptomatic malaria incidence in older children.

In summary, in this setting, IPT with dihydroartemisinin–piperaquine given every 4 weeks in young children was safe, well tolerated, and associated with a marked reduction in the burden of malaria compared with dihydroartemisinin–piperaquine given every 12 weeks; furthermore, this protection was sustained for 1 year after cessation of the intervention. These findings add to a growing body of literature indicating that dihydroartemisinin–piperaquine should be considered as IPT during childhood in settings with high sulfadoxine–pyrimethamine resistance. Future research should focus on mechanisms of immunity and should include pooled analyses of existing studies to improve the precision of estimates of protective efficacy against malaria, evaluations of cost-effectiveness, feasibility studies to assess compliance and effectiveness in real world settings, and additional clinical trials in other epidemiological settings.

Contributors

DVH, MF, GD, and MRK conceived the study with input from MKM, PJ, AK, TDC, TR, and EC. MKM, AK, TC, and GD designed the study and wrote the protocol. MKM and AK coordinated the fieldwork with input from GD and MRK. PO coordinated the laboratory work with input from PJ. BO and NN supervised drug dosing, formulation, preparation of study drugs and placebos, and JO supervised data management. PJ and GD did the statistical analysis with input from MKM, AK, and MRK. All authors reviewed the protocol and approved the manuscript for publication.

Declaration of interests

We declare no competing interests.

Data sharing

Data collected for the study, including individual participant data and data dictionaries defining fields in the datasets, are available on request from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Data and Specimen Hub (DASH) via https://dash.nichd.nih.gov/Resource/Tutorial. Available data include de-identified individual level screening and enrolment data, individual participant data, repeated measures during childhood, repeated doses for vomiting, and corresponding data dictionaries. To access the study protocol, statistical analysis plan, case report forms, and informed consent documents via https://dash.nichd.nih.gov a research data request must be processed. The NICHD DASH Data Access committee reviews all requests to determine that a requester's proposed use of the data is scientifically and ethically appropriate and does not conflict with constraints or informed consent limitations identified by the institutions that submitted the data.

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