In Vivo Efficacy and Tolerability of Artesunate–Azithromycin for the Treatment of *Falciparum* Malaria in Vietnam

Nguyen Chinh Phong,¹ Huynh Hong Quang,² Nguyen Xuan Thanh,¹ Trieu Nguyen Trung,² Bui Dai,¹ G. Dennis Shanks,³ Marina Chavchich,³ and Michael D. Edstein^{3*}

¹Malaria Department, Military Institute of Preventive Medicine, Hanoi, Vietnam; ²Malaria Department, Institute of Malariology, Parasitology and Entomology, Quy Nhon, Vietnam; ³Department of Drug Evaluation, Australian Army Malaria Institute, Brisbane, Australia

Abstract. Safe and effective antimalarial drugs are required for the treatment of pregnant women. We report a 3-day regimen of artesunate (4 mg/kg/day)–azithromycin (25 mg/kg/day) (ASAZ) to be efficacious (polymerase chain reaction–corrected cure rate of 96.7%) and well tolerated in the treatment of *Plasmodium falciparum* malaria in children ($N = 11$) and adults ($N = 19$), in Vietnam in 2010. In comparison, the cure rate for artesunate (4 mg/kg on day 0, 2 mg/kg on days 1–6) was 90.0% in children ($N = 7$) and adults ($N = 23$). Because azithromycin is considered safe in pregnancy, our findings provide further evidence that ASAZ should be evaluated for the treatment of pregnant women with malaria.

Malaria in pregnancy is a serious health problem.¹ Treatment options for malaria in pregnant women, particularly during the first trimester, are limited due to the lack of safety, efficacy, and pharmacokinetic data on antimalarials, including artemisinin-based combination therapies (ACT). The World Health Organization (WHO) recommends the coadministration of quinine and clindamycin (10 mg/kg twice a day) for 7 days during the first trimester.¹ The disadvantage of this regimen is that it causes cinchonism,² and 7-day regimens are associated with poor compliance in an outpatient setting.³ WHO recommends that if quinine–clindamycin is not available or fails to cure, an ACT or artesunate–clindamycin should be used.¹ However, there is a paucity of efficacy and safety data on artesunate–clindamycin, with the only published study in malaria-infected children treated with artesunate (2 mg/kg)–clindamycin (7 mg/kg) twice daily for 3 days producing a cure rate of only 87%.⁴

A potential alternative to artesunate–clindamycin is artesunate–azithromycin (ASAZ). Both clindamycin and azithromycin are broad-acting antibiotics but with different pharmacokinetic properties in pregnant women. Clindamycin has a short elimination half-life of 2–4 hours,⁵ necessitating twice-daily dosing, whereas azithromycin has a longer elimination half-life of 78 hours in pregnant women,⁶ that allows for once-daily dosing. Azithromycin also is well tolerated and considered safe in pregnancy,⁶ and in combination with artesunate could be a safe and effective ACT for the treatment of pregnant women with malaria. Of note, interest in azithromycin has not just been limited to combining with artesunate, but other investigators have been evaluating the antibiotic with chloroquine, piperazine, or sulfadoxine–pyrimethamine for intermittent preventive treatment of malaria in pregnancy.^{7–9}

The objective of the present study was to obtain efficacy and tolerability data of ASAZ in children and adults with uncomplicated *Plasmodium falciparum* malaria in south-central Vietnam. The efficacy of artesunate alone was also assessed to obtain an insight into the susceptibility of artesunate without a partner drug. As this was the first efficacy trial of ASAZ in Vietnam, it was conducted as a pilot study, and was not

powered to detect a statistically significant difference in cure rates between the two treatment regimens. It was considered that 30 evaluable patients in each treatment arm would provide an indication of the efficacy and tolerability of ASAZ, including susceptibility data of artesunate alone at the field site. The study was conducted in ethnic minority patients at Phuoc Chien Commune (Thuan Bac District, Ninh Thuan Province) from May 2010 to October 2010, with sequential recruitment of patients as previously described.¹⁰

Patient inclusion criteria were: age 5–65 years, a blood slide confirmed *P. falciparum* mono-infection with a parasite density of 100–200,000 asexual parasites/ μ L, and tympanic temperature $\geq 38^{\circ}\text{C}$ at the time of enrolment or history of fever during the preceding 24 hours. Exclusion criteria were as follows: patients with severe malaria, antimalarial treatment within the preceding 7 days, mixed plasmodial infection, or pregnant and lactating females. Written informed consent was obtained from each adult patient or from parents or legal guardians of enrolled children. The study was approved by the Vietnam People's Army Military Medicine Department and the Australian Defense Human Research Ethics Committee (approval no. 586/10). The study was registered at the Australian New Zealand Clinical Trials Registry (ACTRN 12610000546055).

Drug administration was based on body weight. Patients in the artesunate arm were administered artesunate (50 mg tablets; Traphaco Joint Stock Company, Hanoi, Vietnam) 4 mg/kg on day 0 followed by 2 mg/kg daily for 6 days. Patients in the ASAZ arm were coadministered artesunate (4 mg/kg) and azithromycin (500 mg Zithromax tablets; Pfizer Italia, Latina, Italy) (25 mg/kg) daily for 3 days. At the time of the study, artesunate monotherapy regimen was still available in the private sector and used where dihydroartemisinin–piperazine was not available.¹¹ Blood films were collected before commencement of treatment and at about every 12 hours after starting treatment until blood films were negative, and then at days 7, 14, 21, and 28 after starting both treatments, with additional collections on days 35 and 42 for the ASAZ group. Patients' tympanic body temperatures were measured before and then at about every 12 hours after starting treatment until their temperature was $< 38^{\circ}\text{C}$. Measurement of parasitemia and parasite and fever clearance times were determined as previously described.¹²

Blood spots on filter paper (3MM Whatman, GE Healthcare, Buckinghamshire, United Kingdom) were collected at the same time as blood smears. Parasite genotyping using polymerase

*Address correspondence to Michael D. Edstein, Australian Army Malaria Institute, Weary Dunlop Drive, Gallipoli Barracks, Enoggera, Brisbane, Queensland 4051, Australia. E-mail: mike.edstein@defence.gov.au

chain reaction (PCR) was done to confirm the plasmodial species¹³ and to differentiate reinfection from recrudescence.¹⁴ Recently, mutations such as C580Y, R539T, and Y439H in the propeller domain of the *PfKelch 13* (Pf3D7_1343700) gene were identified as candidate molecular markers of artemisinin resistance in southeast Asia.¹⁵ K13-propeller gene amplification at positions 449 amino acid to 623 amino acid and sequencing of the 620-base pair portion of the gene were performed on the patient's admission parasites.

Drug tolerability was assessed clinically, and adverse events were recorded by the physician at the time of each drug administration. No causal association was made with the reported adverse events as it is often difficult to distinguish disease effects from drug effects.¹⁶

The baseline characteristics of the study patients and their clinical and parasitological responses to treatment are shown in Table 1. When combining the responses of children and adults, both treatment regimens promptly reduced fever, with a median fever clearance time of 12 hours. Although the median parasite clearance time was shorter in the artesunate group compared with the ASAZ group (24 hours versus 36 hours), the difference was not statistically significant (Mann-Whitney *U* test, *P* = 0.616). The parasite clearance rate was assessed in 50% (15/30) and 36% (11/30) of patients treated with artesunate and ASAZ, respectively, with ≥ 3 parasite counts using the parasite clearance estimator.¹⁷ The median slope half-life was estimated at 2.7 hours (interquartile range, 2.5–3.2 hours) for artesunate, and at 3.2 hours (2.8–3.3 hours) for ASAZ. The comparable parasite clearance times and rates between the two treatment groups was not unexpected as 4 mg/kg of artesunate was administered on the

first day to both study groups. Furthermore, the parasitological effect of azithromycin is not immediate as the drug has a delayed death effect in inhibiting the next generation of parasites.¹⁸

Five patients in the artesunate group presented with a recurrence of malaria (*P. falciparum*: *N* = 3 and *Plasmodium vivax*: *N* = 2) by day 28 of follow-up. In contrast, there were two recurrences of malaria (*P. falciparum*: *N* = 1 and *P. vivax*: *N* = 1) in the ASAZ group by day 28 of follow-up, with a further *P. falciparum* infection diagnosed by day 35. The PCR-corrected cure rate at day 28 was 90.0% (27 of 30) for the artesunate group and 100% cure rate for the ASAZ group. The overall, PCR-corrected cure rate by day 42 for the ASAZ group was 96.7% (29 of 30), with one recrudescence diagnosed on day 35.

The two treatment regimens were well tolerated, with fever, headache, nausea, and tiredness being the most common adverse events reported before treatment (Table 2). These symptoms declined markedly 24 hours after starting treatment, with most patients free of adverse events within 2 days of commencing treatment. Only two patients reported transient mild diarrhea or abdominal pain on ASAZ and none on artesunate alone. The gastrointestinal disturbances were probably azithromycin related as these adverse events were also experienced in healthy Vietnamese volunteers administered the same 3-day ASAZ regimen.¹⁹

The high efficacy and good tolerability of ASAZ reported in the present study further supports earlier studies in Bangladesh²⁰ and Thailand²¹ that showed a 3-day course of azithromycin (30 mg/kg/day)–artesunate (4 mg/kg/day) to be effective (PCR-corrected cure rate > 92%) in curing uncomplicated

TABLE 1
Baseline characteristics of study participants and clinical and parasite response to treatment with AS or ASAZ for uncomplicated falciparum malaria

Characteristics	AS		ASAZ	
	Children	Adults	Children	Adults
No. of patients	7	23	11	19
No. of males (%)	4 (57%)	13 (57%)	7 (64%)	14 (74%)
Mean age, years (SD)	9.8 (2.6)	27.3 (9.8)	9.8 (3.2)	27.1 (13.9)
Mean weight, kg (SD)	26.0 (5.5)	46.0 (9.9)	28.1 (7.7)	46.4 (6.0)
Mean temperature, °C (SD)	38.0 (1.3)	38.6 (1.2)	38.5 (0.8)	38.7 (1.1)
No. (%) of patients with temp $\geq 38^{\circ}\text{C}$	3 (43%)	14 (61%)	7 (64%)	14 (74%)
Geometric mean (range) asexual <i>Plasmodium falciparum</i> /μL	42,161 (10,664–113,024)	22,500 (1,896–126,572)	39,993 (18,898–129,500)	23,666 (1,904–145,064)
Fever clearance time, (hours), median (range)	24*	12 (12–36)	12 (12–24)	12 (12–24)
Parasite clearance time (hours), median (range)	36 (24–48)	24 (12–48)	24 (12–48)	36 (12–48)
No. of patients with recurrent parasitemia by day 28 of follow-up	2	3	0	2
No. of patients with recurrent parasitemia by day 42 of follow-up	ND	ND	1	2
Treatment failure after PCR genotyping	2	1	1	0
Geometric mean (range) dose (mg/kg)	19.9 (16.7–23.0) over 7 days	21.6 (15.4–19.6) over 7 days	13.2 (10.5–15.0) for AS plus 77.1 (52.3–90.0) for AZ over 3 days	13.5 (10.9–14.7) for AS plus 74.6 (62.5–83.3) for AZ over 3 days

AS = artesunate; ASAZ = artesunate–azithromycin; AZ = azithromycin; ND = not determined; PCR = polymerase chain reaction; SD = standard deviation.

*All three children were afebrile at 24 hours after starting artesunate treatment.

TABLE 2
Adverse events before and after treatment of patients with artesunate or ASAZ for uncomplicated falciparum malaria

Artesunate	Before treatment	24 hours after 1st dose	24 hours after 2nd dose
Fever	56.6% (17/30)	3.3% (1/30)	NR
Headache	66.7% (20/30)	50.0% (15/30)	NR
Tiredness	73.0% (22/30)	43.0% (13/30)	NR
Nausea	33.3% (10/30)	NR	NR
Vomiting	16.7% (5/30)	NR	NR
Diarrhea	NR	NR	NR
Abdominal pain	NR	NR	NR
ASAZ	Before treatment	24 hours after 1st dose	24 hours after 2nd dose
Fever	70.0% (21/30)	NR	NR
Headache	83.3% (25/30)	56.6% (17/30)	3.3% (1/30)
Tiredness	96.6% (29/30)	36.6% (11/30)	NR
Nausea	40.0% (12/30)	10.0% (3/30)	NR
Vomiting	6.6% (2/30)	NR	NR
Diarrhea	NR	3.3% (1/30)	NR
Abdominal pain	3.3% (1/30)	3.3% (1/30)	NR

ASAZ = artesunate–azithromycin; NR = not reported.

P. falciparum malaria in adults and children. However, a lower dosage of azithromycin (20 mg/kg) coadministered with artesunate (4 mg/kg) given daily for 3 days in Tanzania²² and Thailand²¹ was less efficacious (PCR-corrected cure rate < 89%), emphasizing the importance of adequate azithromycin dosing. The azithromycin dose of 25 mg/kg/day selected in the present study was considered the maximum acceptable dose based on our previous study in healthy Vietnamese volunteers.¹⁹

Prolongation of parasite clearance times (> 72 hours) reported in studies from western Cambodia²³ and south Vietnam²⁴ with artesunate alone was not evident in the present study, with no patients having parasites present on day 3 after starting treatment, suggesting that the parasite populations were susceptible to artesunate at the time of the study. This was further confirmed with a lack of polymorphism in the *PfKelch 13* gene of falciparum parasites sampled from the patients. However, after the present study, artemisinin resistance has been reported in four provinces of Vietnam (Binh Phuoc, Dak Nong, Gia Lai, and Quang Nam).²⁵ Because three of these provinces are located within 250 km from Ninh Thuan Province, it is important that longitudinal monitoring for artemisinin resistance is performed to determine the evolution and spread of resistance in Vietnam.

Although the number of study patients was too small for us to provide definitive efficacy and tolerability data for ASAZ, the cure rate of 96.7% is highly promising. In contrast to the twice-daily dosing with artesunate–clindamycin, the daily regimen of ASAZ may provide improved efficacy and compliance. However, caution needs to be exercised in that the trial was not conducted in a highly artemisinin-resistant area, and therefore, ASAZ may not be as effective in regions with artemisinin resistance. Since WHO aims at a cure rate > 95% for the selection of future ACTs for the treatment of uncomplicated falciparum malaria,¹ our findings in Vietnam and those from other studies^{20,21} suggest that ASAZ is worthy of further investigation in treating falciparum malaria in pregnant women.

Received February 25, 2016. Accepted for publication April 4, 2016.

Published online May 23, 2016.

Acknowledgments: This study was carried out under the auspices of the Vietnam Australia Defence Malaria Project, a defence cooperation between the Vietnam People's Army and the Australian Defence Force. We thank the Australian Defence Force International Policy

Division for financial support. We are grateful to Ivor Harris and Scott Smith (both WHO-certified Level 1 malaria microscopists) in providing quality assurance of the microscopy analysis, and Vu Huy Chien and Henry Simila for PCR analysis.

Disclaimer: The opinions expressed are those of the authors and do not necessarily reflect those of the Australian Defence Organisation or any extant policy.

Authors' addresses: Nguyen Chinh Phong, Nguyen Xuan Thanh, and Bui Dai, Malaria Department, Military Institute of Preventive Medicine, Hanoi, Vietnam, E-mails: mp.impe@gmail.com, nxthanhvspdq@yahoo.com, and buidai2003@yahoo.com. Huynh Hong Quang and Trieu Nguyen Trung, Malaria Department, Institute of Malariology, Parasitology and Entomology, Quy Nhon, Vietnam, E-mails: huynhquangimpe@yahoo.com and trieutrung@dn.vnn.vn. G. Dennis Shanks, Marina Chavchich, and Michael D. Edstein, Department of Drug Evaluation, Australian Army Malaria Institute, Brisbane, Australia, E-mails: dennis.shanks@defence.gov.au, marina.chavchich@defence.gov.au, and mike.edstein@defence.gov.au.

REFERENCES

1. World Health Organization, 2015. *Guidelines for the Treatment of Malaria*, 3rd edition. Geneva, Switzerland: World Health Organization. Available at: <http://www.who.int/malaria/publications/atoz/9789241549127/en/>. Accessed March 17, 2016.
2. Pukrittayakamee S, Chantra A, Vanijanonta S, Clemens R, Looareesuwan S, White NJ, 2000. Therapeutic responses to quinine and clindamycin in multidrug-resistant falciparum malaria. *Antimicrob Agents Chemother* 44: 2395–2398.
3. Fungladda W, Honrado ER, Thimasarn K, Kitayaporn D, Karbwang J, Kamolratanakul P, Masngammueg R, 1998. Compliance with artesunate and quinine + tetracycline treatment of uncomplicated falciparum malaria in Thailand. *Bull World Health Organ* 76 (Suppl 1): 59–66.
4. Ramharther M, Oyakhrome S, Klein Klouwenberg P, Adegnika AA, Agnandji ST, Missinou MA, Matsiegui PB, Mordmuller B, Borrmann S, Kun JF, Lell B, Krishna S, Graninger W, Issifou S, Kremsner PG, 2005. Artesunate-clindamycin versus quinine-clindamycin in the treatment of *Plasmodium falciparum* malaria: a randomized controlled trial. *Clin Infect Dis* 40: 1777–1784.
5. Lell B, Kremsner PG, 2002. Clindamycin as an antimalarial drug: review of clinical trials. *Antimicrob Agents Chemother* 46: 2315–2320.
6. Salman S, Rogerson SJ, Kose K, Griffin S, Gomorai S, Baiwog F, Winmai J, Kandai J, Karunajeewa HA, O'Halloran SJ, Siba P, Ilett KF, Mueller I, Davis TM, 2010. Pharmacokinetic properties of azithromycin in pregnancy. *Antimicrob Agents Chemother* 54: 360–366.

7. Chandra RS, Orazem J, Ubben D, Duparc S, Robbins J, Vandenbroucke P, 2013. Creative solutions to extraordinary challenges in clinical trials: methodology of a phase III trial of azithromycin and chloroquine fixed-dose combination in pregnant women in Africa. *Malar J* 12: 122.
8. Moore BR, Benjamin JM, Auyeung SO, Salman S, Yadi G, Griffin S, Page-Sharp M, Batty KT, Siba PM, Mueller I, Rogerson SJ, Davis TM, 2016. Safety, tolerability and pharmacokinetic properties of co-administered azithromycin and piperazine in pregnant Papua New Guinean women. *Br J Clin Pharmacol*. doi:10.1111/bcp.12910. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26889763>. Accessed March 17, 2016.
9. Unger HW, Ome-Kaius M, Wangnapi RA, Umbers AJ, Hanieh S, Suen CS, Robinson LJ, Rosanas-Urgell A, Wapling J, Lufele E, Kongs C, Samol P, Sui D, Singirok D, Bardaji A, Schofield L, Menendez C, Betuela I, Siba P, Mueller I, Rogerson SJ, 2015. Sulphadoxine-pyrimethamine plus azithromycin for the prevention of low birthweight in Papua New Guinea: a randomised controlled trial. *BMC Med* 13: 9.
10. Thanh NX, Trung TN, Phong NC, Quang HH, Dai B, Shanks GD, Chavchich M, Edstein MD, 2012. The efficacy and tolerability of artemisinin-piperazine (Artequick[®]) versus artesunate-amodiaquine (Coarsucam[™]) for the treatment of uncomplicated *Plasmodium falciparum* malaria in south-central Vietnam. *Malar J* 11: 217.
11. Thanh NV, Toan TQ, Cowman AF, Casey GJ, Phuc BQ, Tien NT, Hung NM, Biggs BA, 2010. Monitoring for *Plasmodium falciparum* drug resistance to artemisinin and artesunate in Binh Phuoc Province, Vietnam: 1998–2009. *Malar J* 9: 181.
12. Thanh NX, Trung TN, Phong NC, Thien NX, Dai B, Shanks GD, Chavchich M, Edstein MD, 2009. Open label randomized comparison of dihydroartemisinin-piperazine and artesunate-amodiaquine for the treatment of uncomplicated *Plasmodium falciparum* malaria in central Vietnam. *Trop Med Int Health* 14: 504–511.
13. Padley D, Moody AH, Chiodini PL, Saldanha J, 2003. Use of a rapid, single-round, multiplex PCR to detect malarial parasites and identify the species present. *Ann Trop Med Parasitol* 97: 131–137.
14. World Health Organization, 2008. *Methods and Techniques for Clinical Trials on Antimalarial Drug Efficacy: Genotyping to Identify Parasite Populations*. Geneva, Switzerland: World Health Organization. Available at: http://apps.who.int/iris/bitstream/10665/43824/1/9789241596305_eng.pdf. Accessed March 17, 2016.
15. Arie F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, Kim S, Duru V, Bouchier C, Ma L, Lim P, Leang R, Duong S, Sreng S, Suon S, Chuor CM, Bout DM, Menard S, Rogers WO, Genton B, Fandeur T, Miotto O, Ringwald P, Le Bras J, Berry A, Barale JC, Fairhurst RM, Benoit-Vical F, Mercereau-Puijalon O, Menard D, 2014. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* 505: 50–55.
16. Price R, van Vugt M, Phaipun L, Luxemburger C, Simpson J, McGready R, ter Kuile F, Kham A, Chongsuphajaisiddhi T, White NJ, Nosten F, 1999. Adverse effects in patients with acute falciparum malaria treated with artemisinin derivatives. *Am J Trop Med Hyg* 60: 547–555.
17. Flegg JA, Guerin PJ, White NJ, Stepniewska K, 2011. Standardizing the measurement of parasite clearance in falciparum malaria: the parasite clearance estimator. *Malar J* 10: 339.
18. Sidhu AB, Sun Q, Nkrumah LJ, Dunne MW, Sacchetti JC, Fidock DA, 2007. In vitro efficacy, resistance selection, and structural modeling studies implicate the malarial parasite apicoplast as the target of azithromycin. *J Biol Chem* 282: 2494–2504.
19. Chinh NT, Quang NN, Anh CX, Thanh NX, Dai B, Birrell GW, Chavchich M, Edstein MD, 2011. Pharmacokinetics and ex vivo antimalarial activity of artesunate-azithromycin in healthy volunteers. *Antimicrob Agents Chemother* 55: 4412–4415.
20. Thriemer K, Starzengruber P, Khan WA, Haque R, Marma AS, Ley B, Vossen MG, Swoboda P, Akter J, Noedl H, 2010. Azithromycin combination therapy for the treatment of uncomplicated falciparum malaria in Bangladesh: an open-label randomized, controlled clinical trial. *J Infect Dis* 202: 392–398.
21. Noedl H, Krudsood S, Chalermratana K, Silachamroon U, Leowattana W, Tangpukdee N, Looareesuwan S, Miller RS, Fukuda M, Jongsakul K, Sriwichai S, Rowan J, Bhattacharyya H, Ohrt C, Knirsch C, 2006. Azithromycin combination therapy with artesunate or quinine for the treatment of uncomplicated *Plasmodium falciparum* malaria in adults: a randomized, phase 2 clinical trial in Thailand. *Clin Infect Dis* 43: 1264–1271.
22. Sykes A, Hendriksen I, Mtove G, Manda V, Mrema H, Rutta B, Mapunda E, Manjurano A, Amos B, Reyburn H, Whitty CJ, 2009. Azithromycin plus artesunate versus artemether-lumefantrine for treatment of uncomplicated malaria in Tanzanian children: a randomized, controlled trial. *Clin Infect Dis* 49: 1195–1201.
23. Dondorp AM, Nosten F, Yi P, Das D, Phyto AP, Tarning J, Lwin KM, Arie F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut K, Imwong M, Chotivanich K, Lim P, Herdman T, An SS, Yeung S, Singhasivanon P, Day NP, Lindegardh N, Socheat D, White NJ, 2009. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 361: 455–467.
24. Hien TT, Thuy-Nhien NT, Phu NH, Boni MF, Thanh NV, Nha-Ca NT, Thai le H, Thai CQ, Toi PV, Thuan PD, Long le T, Dong le T, Merson L, Dolecek C, Stepniewska K, Ringwald P, White NJ, Farrar J, Wolbers M, 2012. In vivo susceptibility of *Plasmodium falciparum* to artesunate in Binh Phuoc Province, Vietnam. *Malar J* 11: 355.
25. World Health Organization, 2014. *World Malaria Report 2014*. Geneva, Switzerland: World Health Organization. Available at: http://www.who.int/malaria/publications/world_malaria_report_2014/en/. Accessed March 17, 2016.