Available online at www.sciencedirect.com
ScienceDirect
Acta Tropica 100 (2006) 185–191
ACTA TROPICA
www.elsevier.com/locate/actatropica

Activity of azithromycin or erythromycin in combination with antimalarial drugs against multidrug-resistant *Plasmodium falciparum in vitro*

S. Nakornchai *, P. Konthiang

Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

Received 25 April 2006; received in revised form 18 August 2006; accepted 24 October 2006
Available online 28 November 2006

Abstract

Azithromycin, an azalide analog of erythromycin was assayed for its *in vitro* activity against multidrug-resistant *Plasmodium falciparum* K1 strain by measuring the ^{3}H-hypoxanthine incorporation. Azithromycin caused inhibitory effects on the parasite growth with IC_{50} and IC_{90} values of 8.4 ± 1.2 μM and 26.0 ± 0.9 μM, respectively. Erythromycin inhibited growth of *P. falciparum* with IC_{50} and IC_{90} values of 58.2 ± 7.7 μM and 104.0 ± 10.8 μM, respectively. The activity of antimalarial drugs in combination with azithromycin or erythromycin against *P. falciparum* K1 were compared. Combinations of chloroquine with azithromycin or erythromycin showed synergistic effects against parasite growth *in vitro*. Combinations of quinine–azithromycin and quinine–erythromycin showed potentiation. Additive effects were observed in mefloquine–azithromycin and mefloquine–erythromycin combinations. Similar results were also produced by pyronaridine in combination with azithromycin or erythromycin. However, artesunate–azithromycin and artesunate–erythromycin combinations had antagonistic effects. The *in vitro* data suggest that azithromycin and erythromycin will have clinical utility in combination with chloroquine and quinine. The worldwide spread of chloroquine-resistant *P. falciparum* might inhibit the ability to treat malaria patients with chloroquine–azithromycin and chloroquine–erythromycin in areas of drug-resistant. The best drug combinations against multidrug-resistant *P. falciparum* are quinine–azithromycin and quinine–erythromycin.

Keywords: Antimalarial drugs; Azithromycin; Erythromycin; Malaria; *Plasmodium falciparum*; Drug resistance

1. Introduction

Some antibiotics have antimalarial activity that can be used for the treatment of malaria. Combination therapy is one method of overcoming the global challenge of drug-resistance *Plasmodium falciparum* malaria. Antibiotics are used in combination with antimalarial drug against multidrug-resistant *P. falciparum*. Combination of the tetracycline group of antibiotics with quinine and mefloquine has been used successful in Southeast Asia to treat multidrug-resistant *P. falciparum* cases (Watt et al., 1992; Looareesuwan et al., 1994a). The artesunate–tetracycline combination has been shown to be a useful alternative (Duarte et al., 1996; Looareesuwan et al., 1994b). The drugs of the tetracycline group should not be used in pregnant women and young children.

Quinine–clindamycin has been shown to be effective in pregnancy (Mc Gready et al., 2001). Clindamycin is used in combination with either chloroquine or quinine to treat children with uncomplicated falciparum malaria (Kremsner et al., 1994). Side effects of clindamycin are

* Corresponding author. Fax: +66 2 354 7174.
E-mail address: scsnk@mahidol.ac.th (S. Nakornchai).

0001-706X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2006.10.008

diarrhea and colitis. New combinations that are safe and effective against drug-resistant *P. falciparum* in children and pregnant women are clearly needed.

Erythromycin has been shown to have antimalarial activity against *P. falciparum in vitro* and *Plasmodium berghei in vivo* (Gingras and Jensen, 1992; Gingras and Jensen, 1993). Azithromycin is a semisynthetic derivative of erythromycin that differs from erythromycin by possessing a methyl-substituted nitrogen in the macrolide ring. This alteration allows for greater bioavailability, increased tissue penetration and longer elimination half-life compared with those of erythromycin (Dunn and Barradell, 1996). Azithromycin has antimalarial activity against *P. falciparum in vitro*, *P. berghei* and *Plasmodium yoelii in vivo* (Gingras and Jensen, 1992; Gingras and Jensen, 1993; Puri and Singh, 2000). The clinical trial of daily oral azithromycin in adults is effective for prevention of *Plasmodium vivax* malaria (Heppner et al., 2005). Azithromycin is approved for the use in children (Zithromax product information, 1999; Pfizer Inc., New York), and is safe for pregnant women (Gray et al., 2001; Wawer et al., 1999). The objective of this *in vitro* study is to determine the best drug combination between azithromycin or erythromycin and standard antimalarial drugs against multidrug-resistant *P. falciparum* that will be clinically useful.

2. Materials and methods

2.1. Methods for testing drug combination on the P. falciparum growth

Multidrug-resistant *P. falciparum* K1 strain was obtained from an infected individual in Kanchanaburi province, Thailand. They routinely can be maintained in human group O red blood cells in a medium RPMI 1640 supplemented with 10% human serum using the candle jar method (Trager and Jensen, 1976). Antimalarial drugs in combination with azithromycin or erythromycin (Fig. 1) were tested against *P. falciparum in vitro* by ^{3}H-hypoxanthine incorporation (Desjardin et al., 1979). In the experiment, 200 μl of 0.5% parasitized cells, 3% red blood cells suspension and 25 μl of drug solution (individually or in combination) were placed in 96-well plates in triplicate. After incubating at 37 °C for 48 h, 25 μl of ^{3}H-hypoxanthine was added to a final concentration 1 μCi/ml and further incubated at 37 °C for 48 h. Red blood cells were harvested using an automated sample harvester and counted for radioactivity, which was incorporated into the parasites with a Beckman liquid scintillation counter model LS-1801 (Beckman Instruments Inc.). ^{3}H-hypoxanthine uptake was assayed in the presence of both a single drug and a combination of antimalarial drug with azithromycin or erythromycin, and was expressed as percentage of control. The 50% inhibitory concentration (IC_{50}) values, defined as the drug concentration required for 50% reduction of ^{3}H uptake by parasites as compared to the control (without the drugs), were determined from the dose–response curve. The antimalarial drug IC_{50} for various antibiotic concentrations and the antibiotic IC_{50} for various antimalarial drug concentrations were determined and used to construct the isobologram (Berenbaum, 1978).

3. Results

3.1. Effects of antimalarial drugs and antibiotic combinations on the P. falciparum growth

Multidrug-resistant *P. falciparum* K1 growth were inhibited by azithromycin with IC_{50} and IC_{90} values of 8.4 ± 1.2 μM and 26.0 ± 0.9 μM, respectively. The IC_{50} and IC_{90} values for erythromycin against

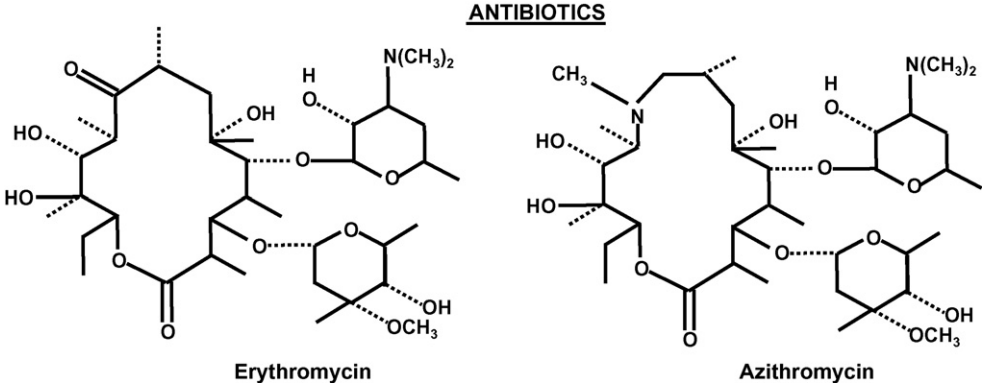

Fig. 1. Structures of erythromycin and azithromycin.

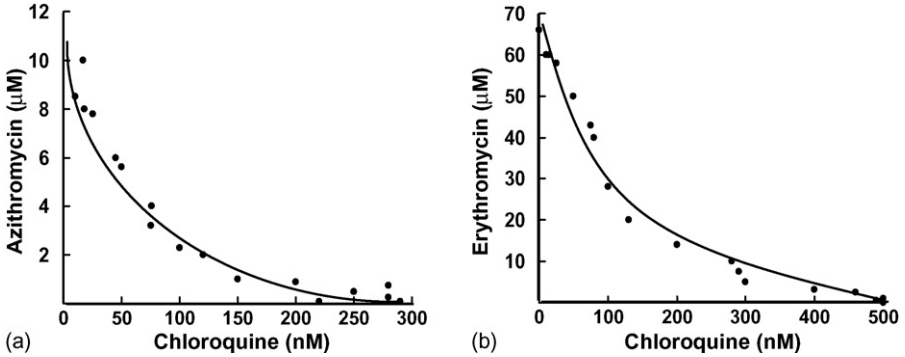

Fig. 2. Isobologram of chloroquine in combination with (a) azithromycin against *Plasmodium falciparum* K1 and (b) erythromycin against *P. falciparum* K1.

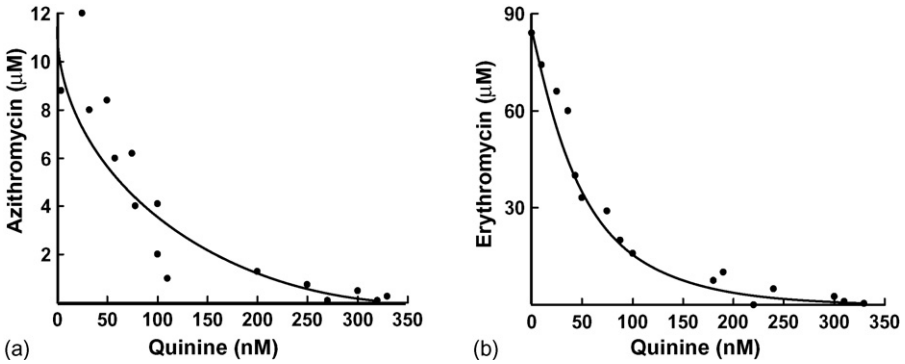

Fig. 3. Isobologram of quinine in combination with (a) azithromycin against *P. falciparum* K1 and (b) erythromycin against *P. falciparum* K1.

P. falciparum K1 were $58.2 \pm 7.7\,\mu M$ and $104.0 \pm 10.8\,\mu M$, respectively. To determine the effect of drug combinations between antimalarial drugs and azithromycin or erythromycin, isobolograms were constructed. The isobologram of an additive combination of two agents lies on a straight line. The isobologram of synergistic agents is concave. With antagonistic agents, the isobologram is convex. Combinations of chloroquine with azithromycin or erythromycin showed synergistic effects against *P. falciparum* growth in culture (Fig. 2a and b). Combination of quinine with azithromycin or erythromycin also showed synergistic effects (Fig. 3a and b). Additive effects were observed between mefloquine with azithromycin or with erythromycin (Fig. 4a and b). Combinations of pyronaridine–azithromycin and of pyronaridine–erythromycin showed similar effects

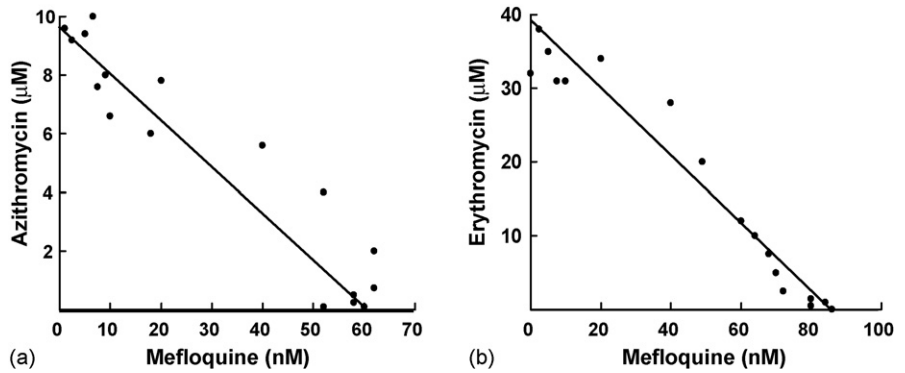

Fig. 4. Isobologram showing effect of mefloquine in combination with (a) azithromycin against *P. falciparum* K1 and (b) erythromycin against *P. falciparum* K1.

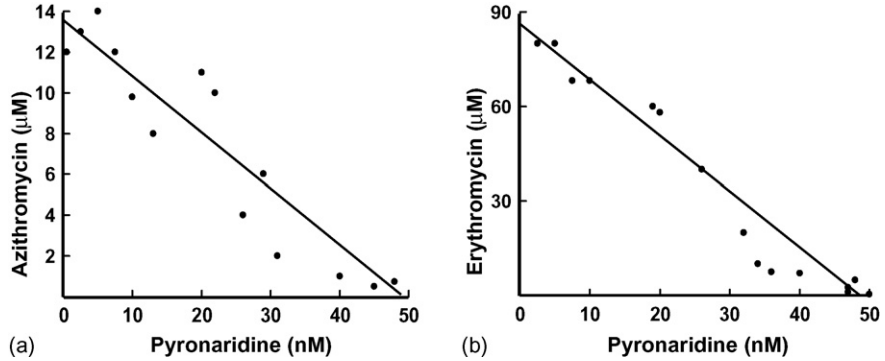

Fig. 5. Isobologram showing effect of pyronaridine in combination with (a) azithromycin against *P. falciparum* K1 and (b) erythromycin against *P. falciparum* K1.

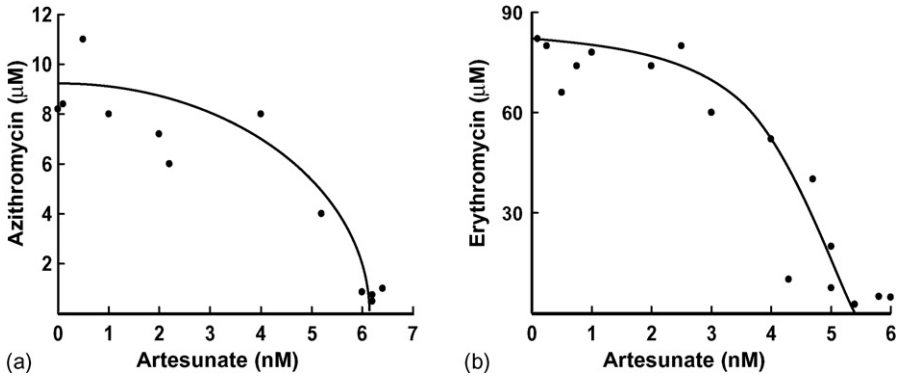

Fig. 6. Isobologram of artesunate in combination with (a) azithromycin against *P. falciparum* K1 and (b) erythromycin against *P. falciparum* K1.

(Fig. 5a and b). However artesunate–azithromycin and artesunate–erythromycin combinations had antagonistic effects (Fig. 6a and b).

4. Discussion

The most common antibiotics used against malaria are tetracycline and doxycycline. A standard treatment for patients with infections by chloroquine-resistant *P. falciparum* strains is a 7-day course of quinine–tetracycline combinations (Bunnag et al., 1996). Quinine–doxycycline and chloroquine–doxycycline were effective in treating chloroquine-resistant uncomplicated falciparum malaria patients (Taylor et al., 2001). Drug of tetracycline class however cannot be used in pregnant women and young children because of their interference with skeletal and tooth development.

In this study, *P. falciparum* K1 growth was inhibited by azithromycin with IC_{50} and IC_{90} values of 8.4 ± 1.2 μM and 26.0 ± 0.9 μM, respectively. The IC_{50} and IC_{90} values of erythromycin against *P. falciparum* K1 were 58.2 ± 7.7 μM and 104.0 ± 10.8 μM, respectively. The effective concentrations EC_{50} and EC_{90} of azithromycin against fresh isolates of *P. falciparum* in Thailand were 29.3 μM and 77.1 μM, respectively (Noedl et al., 2001). In the prersent study, azithromycin was seven-fold more potent than erythromycin against multidrug-resistant *P. falciparum* K1 in culture. Previous *in vitro* study had indicated that azithromycin is 10-fold more potent than erythromycin against chloroquine-resistant *P. falciparum* FCR $_{3TC}$ isolates (Gingras and Jensen, 1992). Against the chloroquine-resistant *P. berghei* and *P. yoelii* in mice, azithromycin was 60-fold and 31-fold more potent than erythromycin, respectively (Gingras and Jensen, 1993; Puri and Singh, 2000).

The data presented in Fig. 2a and b indicates that chloroquine in combination with azithromycin or erythromycin for 96 h incubation acts synergistically against multidrug-resistant *P. falciparum* K1. The chloroquine–azithromycin combinations (incubation time 68 h) revealed a range of activity from additive to synergistic for eight chloroquine-resistant *P. falciparum* isolates (Ohrt et al., 2002). Chloroquine was synergistic with azithromycin for two resistant *P. falciparum* C2A and C2B isolates from Thailand. A

pilot clinical trial showed promising results in treatment of acute uncomplicated *Plasmodiun falciparum* malaria in India with chloroquine in combination with azithromycin (Dunne et al., 2005). Azithromycin had no clinically relevant effect on chloroquine pharmacokinetics (Cook et al., 2006). Previous *in vitro* study had indicated that chloroquine–erythromycin combination showed synergistic effect against chloroquine-resistant *P. falciparum* (Gershon and Howells, 1984). Erythromycin displays synergism with chloroquine against chloroquine-resistant strains of *P. berghei* (Warhurst et al., 1976). Limited clinical trials with erythromycin failed to improve the chloroquine treatment of chloroquine-resistant falciparum malaria in Eastern Thailand (Phillips et al., 1984).

Drug combination experiments of quinine with azithromycin or erythromycin in Fig. 3a and b show synergistic effects against *P. falciparum* K1 *in vitro*. This result similars that of a previous study, which demonstrated synergism between quinine–azithromycin combination against chloroquine-resistant *P. falciparum* C2A and C2B isolates from Thailand (Ohrt et al., 2002). Quinine was used in combination with azithromycin for clinical trials in the treatment of uncomplicated falciparum malaria in Thailand (Miller et al., 2002). Treatment of uncomplicated *P. falciparum* malaria patients with quinine–azithromycin combinations for 3 days appeared to be better tolerated than 7-day combination of quinine with doxycycline (Miller et al., 2006). The maximum concentration of azithromycin in serum is 10 mg/l after a 4 g of azithromycin (Luke et al., 1996), which is the IC_{90} level for azithromycin against *P. falciparum* K1 *in vitro*. The maximum serum concentration of erythromycin after 1 g of erythromycin is 33 mg/l (Sietzen, 1982), which is the IC_{90} level of erythromycin against *P. falciparum* K1 *in vitro*. Our study demonstrates that quinine–azithromycin and quinine–erythromycin may serve as an alternative for tetracycline and doxycycline. The advantage in substituting azithromycin for tetracycline in combination therapy is not only with respect to potential for use in children and pregnant women, but also with respect to its safety and tolerability (Sadiq et al., 1995; Miller et al., 2006). Azithromycin is a potential, chemotherapeutic agent which posses antimalarial activity and favourable pharmacokinetic properties. The side effects of azithromycin are headache, mild abdominal pain that occurs in 3–5% of patients, which lasted almost a day (Anderson et al., 1995). Azithromycin has the ability to inhibit bacterial protein synthesis. Recently specific inhibition of protein synthesis by azithromycin has been demonstrated in the related apicomplexan protozoan *Toxoplasma gondii* (Blais et al., 1993). Azithromycin, being a dibasic amine, accumulates within fibroblasts and phagocytic lysosomes to a greater degree than does the monobasic macrolides such as erythromycin (Gladue et al., 1989; Gladue and Snider, 1990). Azithromycin is a weak base. It may, like chloroquine raise the pH of the digestive vacuole and thereby prevent the organism from managing the accumulating hematin. Azithromycin has been shown to increase the intracellular accumulation of doxorubicin in adriamycin-resistant human myclogenous leukemia cells (Asakura et al., 2004). The mechanism of malarial chloroquine resistance may be similar to the mechanism of multidrug-resistant phenotype of mammalian tumor cells (Bray and Ward, 1998). The resistance-reversing activity of verapamil is associated with the increasing accumulation of chloroquine by the resistant parasites (Krogstad et al., 1987). This phenomenon of resistance reversal by verapamil is also key features of drug-resistance in mammalian cells.

When combinations of mefloquine–azithromycin and mefloquine–erythromycin were tested, the effects were additive. Mefloquine in combination with tetracycline has proven to be equally effective and be less toxic than quinine plus tetracycline for treatment of uncomplicated falciparum malaria (Looareesuwan et al., 1994a). Although synergistic effect is beneficial to the patient, it is not essential for a successful drug combination. Some nonsynergistic combinations can be beneficial for chemotherapy such as quinine or mefloquine in combination with tetracycline, which showed additive effects *in vitro* (Watt et al., 1992; Looareesuwan et al., 1994a). Combinations of pyronaridine–azithromycin and pyronaridine–erythromycin have also shown additive effects.

Artesunate–azithromycin and artesunate–erythromycin combinations have antagonistic effects. The cure rate of artemether–azithromycin regimen are lower than that of artemether–doxycycline regimen (Na-Bangchang et al., 1996). Azithromycin is not useful in preventing recrudescences when given as a short course in combination with either artesunate or artemether (De Vries et al., 1999; Na-Bangchang et al., 1996). A combination of dihydroartemisinin and azithromycin is less effective than dihydroartemisinin and mefloquine for treatment of multidrug-resistant falciparum malaria (Krudsood et al., 2002).

The worldwide spread of *P. falciparum* resistant to chloroquine in Southeast Asia, South America and in Africa may inhibited the ability to treat the disease with combinations of chloroquine with azithromycin or erythromycin in these endemic areas. We conclude that the best drug combinations against multidrug-

resistant *P. falciparum* are quinine–azithromycin and quinine–erythromycin. Azithromycin has greater bioavialability, tissue penetration and longer elimination half-life compared with those erythromycin (Dunn and Barradell, 1996). Erythromycin is widely available at low cost in malaria endemic zones. Synergism between quinine–azithromycin and quinine–erythromycin indicate that these may be promising combinations in areas with high prevalence of multidrug-resistance. A pilot clinical trial showed promising result in the treatment of uncomplicated *P. falciparum* malaria patients with quinine–azithromycin combinations (Miller et al., 2006). The clinical effect of azithromycin and erythromycin on quinine pharmacokinetics in human should be further investigated.

References

Anderson, S.L., Berman, J., Kuschner, R., Wesche, D., Magill, A., Wellde, B., Schneider, I., Dunne, M., Schuster, B.G., 1995. Prophylaxis of *Plasmodium falciparum* malaria with azithromycin administered to volunteers. Ann. Intern. Med. 123, 771–773.

Asakura, E., Nakayama, H., Sugie, M., Zhao, Y.L., Nadai, M., Kitaichi, K., Shimizu, A., Miyoshi, M., Takagi, K., Hasegawa, T., 2004. Azithromycin reverses anticancer drug resistance and modifies hepatobiliary excretion of doxorubicin in rats. Eur. J. Pharmacol. 484, 333–339.

Berenbaum, M.C., 1978. A method for testing synergy with any number of agents. J. Infect. Dis. 137, 122–130.

Blais, J., Garneau, V., Chamberland, S., 1993. Inhibition of *Toxoplasma gondii* protein synthesis by azithromycin. Antimicrob. Agents. Chemother. 37, 1701–1703.

Bray, P.G., Ward, S.A., 1998. A comparison of the phenomenology and genetics of multidrug resistance in *Plasmodium falciparum*. Pharmacol. Ther. 77, 1–28.

Bunnag, D., Karbwang, J., Na-Bangchang, K., Thanavibul, A., Chittamas, S., Harinasuta, T., 1996. Quinine-tetracycline for multidrug-resistant falciparum malaria. Southeast Asian J. Trop. Med. Publ. Health 27, 15–18.

Cook, J.A., Randinitis, E.J., Bramson, C.R., Wesche, D.L., 2006. Lack of a pharmacokinetic interaction between azithromycin and chloroquine. Am. J. Trop. Med. Hyg. 74, 407–412.

Desjardin, R.E., Canfield, C.J., Haynes, J.D., Chulay, J.D., 1979. Quantitative assessment of antimalarial activity *in vitro* by semiautomated microdilution technique. Antimicrob. Agents Chemother. 16, 710–718.

De Vries, P.J., Hung, L.N., Thuy, L.T.D., Long, H.P., Nam, N.V., Anh, T.K., 1999. Short course of azithromycin–artesunate against falciparum malaria: no full protection against recrudescence. Trop. Med. Intern. Health 4, 407–408.

Duarte, E.C., Fontes, C.J., Gyorkos, T.W., Abrahamowicz, A., 1996. Randomized controlled trial of artesunate plus tetracycline versus standard treatment (quinine plus tetracycline) for uncomplicated *Plasmodium falciparum* malaria in Brazil. Am. J. Trop. Med. Hyg. 54, 197–202.

Dunn, C.J., Barradell, L.B., 1996. Azithromycin: a review of its pharmacological properties and use as a 3-day therapy in respiratory tract infection. Drugs 51, 483–505.

Dunne, M.W., Singh, N., Shukla, M., Valecha, N., Bhattacharyya, P.C., Dev, V., Patel, K., Mohapatra, M.K., Lakhani, J., Benner, R., Lele, C., Patki, K., 2005. A multicenter study of azithromycin alone and in combination with chloroquine, for the treatment of acute uncomplicated *Plasmodium falciparum* malaria in India. J. Infect. Dis. 191, 1582–1588.

Gershon, P.D., Howells, R.E., 1984. Combination of antibiotics erythromycin and tetracycline with three standard antimalarials against *Plasmodium falciparum in vitro*. Ann. Trop. Med. Parasitol. 78, 1–11.

Gingras, B.A., Jensen, J.B., 1992. Activity of azithromycin (CP-62,993) and erythromycin against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum in vitro*. Am. J. Trop. Med. Hyg. 47, 378–382.

Gingras, B.A., Jensen, J.B., 1993. Antimalarial activity of azithromycin and erythromycin against *Plasmodium berghei*. Am. J. Trop. Med. Hyg. 49, 101–105.

Gladue, R.P., Bright, G.M., Isaacson, R.E., Newborg, M.F., 1989. *In vitro* and *in vivo* uptake of azithromycin (CP-62,993) by phagocytic cells: possible mechanism of delivery and release at sites of infection. Antimicrob. Agents Chemother. 33, 277–282.

Gladue, R.P., Snider, M.E., 1990. Intracellular accumulation of azithromycin by cultured human fibroblasts. Antimicrob. Agents Chemother. 34, 1056–1060.

Gray, R.H., Wabwire-Mangen, F., Kigozi, G., Sewankambo, N.K., Serwadda, D., Moulton, L.H., Quinn, T.C., O'Brien, K.L., Meechau, M., et al., 2001. Randomized trial of presumptive sexually transmitted disease therapy during pregnancy in Rakai, Uganda. Am. J. Obstet. Gynecol. 185, 1209–1217.

Heppner Jr., D.G., Walsh, D.S., Uthaimongkol, N., Tang, D.B., Tulyayon, S., Permpanich, B., Wimonwattrawatee, T., Chuanak, N., Laoboonchai, A., et al., 2005. Randomized controlled double-blind trial of daily oral azithromycin in adults for prophylaxis of *Plasmodium vivax* malaria in Western Thailand. Am. J. Trop. Med. Hyg. 73, 842–849.

Kremsner, P.G., Winkler, S., Brandts, C., Neifer, S., Bienzle, U., Graninger, W., 1994. Clindamycin in combination with chloroquine or quinine is an effective therapy for uncomplicated *Plasmodium falciparum* malaria in children from Gabon. J. Infect. Dis. 169, 467–470.

Krogstad, D.J., Gluzman, I.Y., Kyle, D.E., Oduola, A.M.J., Martin, S.K., Milhous, W.K., Schlesinger, P.H., 1987. Efflux of chloroquine from *Plasmodium falciparum*: mechanism of chloroquine resistance. Science 238, 1283–1285.

Krudsood, S., Buchachart, K., Chalermrut, K., Charusabha, C., Treeprasertsuk, S., Haoharn, O., Duangdee, C., Looareesuwan, S., 2002. A comparative clinical trial of combination of dihydroartemisinin plus azithromycin and dihydroartemisinin plus mefloquine for treatment of multidrug-resistant falciparum malaria. Southeast Asian J. Trop. Med. Hyg. 33, 525–531.

Looareesuwan, S., Vanijanonta, S., Viravan, C., Wilairatana, P., Charoenlarp, P., Lasserre, R., Canfield, C., Kyle, D.E., Webster, H.K., 1994a. Randomised trial of mefloquine–tetracycline and quinine–tetracycline for acute uncomplicated falciparum malaria. Acta Trop. 57, 47–53.

Looareesuwan, S., Viravan, C., Vanijanonta, S., Wilairatana, P., Charoenlarp, P., Canfield, C.J., Kyle, D.E., 1994b. Randomized trial of mefloquine–doxycycline and artesunate–doxycycline for the treatment of acute uncomplicated falciparum malaria. Am. J. Trop. Med. Hyg. 50, 784–789.

Luke, D.R., Foulds, G., Cohen, S.F., Levy, B., 1996. Safety, toleration, and pharmacokinetics of intravenous azithromycin. Antimicrob. Agents Chemother. 40, 2577–2581.

Mc Gready, R., Samuel, C.T., Villegae, L., Brockman, A., Van Vugt, M., Looareesuwan, S., White, N.J., Nosten, F., 2001. Randomized comparison of quinine–clindamycin versus artesunate in the treatment of falciparum malaria in pregnancy. Trans. R. Soc. Trop. Med. Hyg. 95, 651–656.

Miller, R.S., McDaniel, P., Wongsrichanalai, C., 2002. Azithromycin–quinine combination therapy for the treatment of uncomplicated falciparum malaria in Thailand. In: Program and Abstracts of the American Society of Tropical Medicine and Hygeine, Denver, CO, Abstract 567. American Society for Tropical Medicine and Hygeine, Northbrook, IL.

Miller, R.S., Wongsrichanalai, C., Buathong, N., Mc Daniel, P., Walsh, D.S., Knirsch, C., Ohrt, C., 2006. Effective treatment of uncomplicated *Plasmodium falciparum* malaria with azithromycin–quinine combinations: a randomized dose-ranging study. Am. J. Trop. Med. Hyg. 74, 401–406.

Na-Bangchang, K., Kanda, T., Tipawangso, P., Thanavibul, A., Suprakob, K., Ibrahim, M., Wattanagoon, Y., Karbwang, J., 1996. Activity of artemether–azithromycin versus artemether–doxycycline in the treatment of multiple drug resistant falciparum malaria. Southeast Asian J. Trop. Med. Publ. Health 27, 522–525.

Noedl, H., Werndorfer, W.H., Krudsood, S., Wilairatana, P., Kollaritsch, H., Wiedermann, G., Looareesuwan, S., 2001. Antimalarial activity of azithromycin, artemisinin and dihydroartemisinin in fresh isolates of *Plasmodium falciparum* in Thailand. Acta Trop. 80, 39–44.

Ohrt, C., Willingmyre, G.D., Lee, P., Knirsch, C., Milhous, W., 2002. Assessment of azithromycin in combination with other antimalarial drugs against *Plasmodium falciparum in vitro*. Antimicrob. Agents Chemother. 46, 2518–2524.

Phillips, R.E., Looareesuwan, S., Karbwang, J., Warrel, D.A., White, N.J., Kasemsarn, P., Warhurst, D.C., 1984. Failure of chloroquine–erythromycin and chloroquine–tetracycline combination in treatment of chloqroquine-resistant falciparum malaria in Eastern Thailand. Lancet 323, 300–302.

Puri, S.K., Singh, N., 2000. Azithromycin: antimalarial profile against blood and sporozoite-induced infections in mice and monkey. Exp. Parasitol. 94, 8–14.

Sadiq, S.T., Glasgow, K.W., Drakely, C.J., Mabey, D.C.W., Bailey, R.L., Muller, O., Greenwood, B.M., 1995. Effects of azithromycin on malariometric indices in the Gambia. Lancet 346, 881–882.

Sietzen, W., 1982. The pharmacokinetics of 1000 mg erythromycin lactobionate i.v. in patients with respiratory tract infections. Infection 10 (Suppl. 2), S99–S101.

Taylor, W.R., Widjaja, H., Richie, T.L., Basri, H., Ohrt, C., Tjitra, Tanfik, E., Jones, T.R., Kain, K.C., Hoffman, S.L., 2001. Chloroquine/doxycycline combination versus chloroquine alone, and doxycycline alone for treatment of *Plasmodium falciparum* and *Plasmodium vivax* malaria in northeastern Irian Jaya, Indonesia. Am. J. Trop. Med. Hyg. 64, 223–228.

Trager, W., Jensen, J.B., 1976. Human malaria parasites in continuous culture. Science 193, 673–675.

Warhurst, D.C., Robinson, B.L., Peters, W., 1976. The chemotherapy of rodent malaria XXIV. The blood schizontocidal action of erythromycin upon *Plasmodium berghei*. Ann. Trop. Med. Parasitol. 70, 253–258.

Watt, G., Loesuttivibool, L., Shanks, G.D., Boudreau, E.F., Brown, A.E., Pavanand, K., Webster, H.K., Wechgritaya, S., 1992. Quinine with tetracycline for the treatment of drug-resistant falciparum malaria in Thailand. Am. J Trop. Med. Hyg. 47, 108–111.

Wawer, M.J., Sewankambo, N.K., Serwadda, D., Quinn, T.C., Paxton, L.A., Kiwanuka, N., Wabwire-Mangen, F., Li, C., Lutalo, T., et al., 1999. Control of sexually transmitted diseases of AIDS prevention in Uganda: a randomised community trial. Lancet 353, 525–535.