ORIGINAL ARTICLE

Evaluation of the Sensitivity *in vitro* of *Plasmodium Falciparum* and *in vivo* of *Plasmodium Chabaudi* Malaria to Various Drugs and their Combinations

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Summary

K1 strain of *Plasmdoium falciparum* is resistant *in vitro* to chloroquine, pyrimethamine and sulfadoxine. Response of this strain to combinations of antimalarial drugs in the *in vitro* hypoxanthine incorporation test was coupled with that of a line of strain NF54 relatively sensitive to chloroquine and fully sensitive to other antimalarials. Pyrimethamine and sulfadoxine showed potentiative synergism against NF54 and less marked against K1.

Erythromycin and chloroquine showed potentiation, but less marked against NF54. Quinine and clindamycin had an additive effect against NF54 but potentiated against K1. Combinations of chloroquine with quinine or amodiaquine or of amodiaquine with clindamycin or erythromycin showed mild antagonistic or additive effects.

In vivo studies in mice, using the 4-day suppressive test, the AS(3CQ) clone of *Plasmodium chabaudi* was resistant to pyrimethamine and chloroquine but sensitive to sulfadoxine. Similar combinations as above were carried out and their responses were compared between the resistant and sensitive strains. For both strains, the combinations of chloroquine-erythromycin, pyrimethamine-sulfadoxine, quinine-clindamycin showed potentiation; antagonistic effects were observed in chloroquine-amodiaquine combinations whereas when amodiaquine combined with erythromycin the effect was additive. Amodiaquine-clindamycin and chloroquine-quinine combinations have an antagonistic effect against the sensitive strain but additive against the resistant strain.

Key Words: Plasmodium falciparum, Plasmodium chabaudi, Antimalarials

Introduction

Multi-drug resistant *Plasmodium falciparum* infection is prevalent and increasing in many parts of the world[1]. Payne[2] has documented resistance of *Plasmodium falciparum* to chloroquine in most countries where there is transmission. Resistance of pre-erythrocytic stage to pyrimethamine has been reported[3] while Lemnge and Inambao[4] and Kremsner *et al*[5] have observed resistance to amodiaquine. Resistance to sulfadoxine-pyrimethamine has been reported widely in Thailand[6], Indonesia, Papua New Guinea, China and Pakistan[7,8] Brazil[5], Venezuela[9], Africa[10]. Quinine resistance has been reported from Solomon Islands[11], Zambia[12], Ghana[13], Malawi[14], Cameron, Guinea and Senegal[15,16,17]. Resistance to mefloquine has been reported in Thailand[18,19], Tanzania[20] and West Africa[21].

In view of those problems, it is important to monitor as accurately as possible the susceptibility of the

parasites to various drugs, singly and in combinations, both those used previously and currently which are likely to be introduced shortly.

The present work reports herein methods, both *in vitro,* using *Plasmodium falciparum* (chloroquine sensitive and resistant) and *in vivo* with *Plasmodium chabaudi* (chloroquine sensitive and resistant) and their results compared.

Materials and Methods

In vitro assay

Four strains of *Plasmodium falciparum* were used. The chloroquine-sensitive strains were Dutch (NF54)[22] and Honduras[23]. The other two chloroquine-resistant strains were Tanzanian (EAT/LON/1/G30)[24] and Thailand (K1)[25].

Several antimalarial drugs were tested singly as well as in combinations. The single drugs were chloroquine, mefloquine, halofantrine, qinghaosu, amodiaquine, quinine, pyrimethamine, sulfadoxine, erythromycin, clindamycin. The combinations were chloroquine-quinine, chloroquine-amodiaquine, pyrimethamine-sulfadoxine, chloroquine-erythromycin, amodiaquine-erythromycin, amodiaquine-clindamycin and quinine-clindamycin.

The sensitivity of single drug experiment was carried out against the four strains of *Plasmodium falciparum* above whereas the combination studies were tested against NF54 and K1 only.

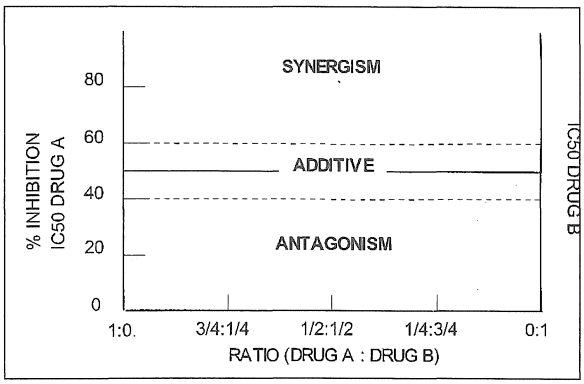
Single drug experiment

The drugs were dissolved in the respective solvents and each drug was kept as a stock solution at 10^{-2}M and stored at -20°C until use.

Determination of 50% inhibition was carried out using the regression of probit percentage inhibition on log-run concentration response curve.

Drug combination experiment

Seven drug combinations were tested as described previously. Preparation of drug mixtures were based on the predetermined 50% inhibition concentration (IC50) values of each drug to be combined as described above. Two drugs were combined in a fixed ratios (1:0, $^3/_4$: $^1/_4$, $^1/_2$: $^1/_2$, $^1/_4$: $^3/_4$, 0 : 1). The effect of the combination was evaluated by mean of an isobologram below:



An isobologram to determine the dynamics of combined drug action *in vivo/vitro*

The values of the percentage inhibitions were not converted to probit but used untransformed. As illustrated the individual percentage inhibition values of each drug pair were plotted on the graph and connected by a line. A simple additive effect (obtained 95% Confidence Limits) would result in the experimental points falling along or near the broken line. Synergism would be indicated by points fall above the line. If the drugs were antagonistic, the points fall between the line and the origin.

In vivo assay

Five male random bred TO mice of 6-8 weeks old and 20-25g weight were used in each treatment group. The 4-day suppressive test[26] was employed on Edinburgh Stabilate 317 (chloroquine sensitive) and AS(3CQ) being chloroquine resistant[27] of *Plasmodium chabaudi.*

The mice received an intravenous inoculum size of approximately 10^7 infected donor erythrocytes on Day 0 (Do). Drugs in distilled water or suspended in Tween 80 were administered intraperitoneally or subcutaneously in equal daily doses for 4 consecutive days, i.e. on Day 0 (Day of Infection), 1, 2, and 3. Parasite levels were estimated in Giemsa stained thin

films from tail blood on Day 4 (D4). The percentage inhibition of parasitaemia seen in individual mouse as a result of the drug activity was then scored. The drug doses were converted to logarithmic values and the inhibition percentages were changed to probit. Drug-dose activity curves could then be drawn to calculate their 50% effective doses (ED50).

Similarly the test was used in the combination studies. The two drugs were administered separately. Each drug dose was based on its ED50 values and the two drugs were combined in a fixed ratios as in the case of *in vitro* experiments. However on Day 4 the percentage inhibitions produced by each combination was determined from the percentage of parasitised cells (combination groups) to the percentage of the controlled parasitised cells. The effect of the combination was also evaluated by means of an isobologram.

Results

A. Single drug experiment

The *in vitro* and *in vivo* activities of antimalarial and antibiotics against their respective strains of parasites are shown in the tables.

Table I
50% inhibitory concentration (IC50) of antimalarial drugs and antibiotics on 2 originally chloroquine-sensitive strains of *Plasmodium falciparum in vitro*

Drug	Strain - IC50μm ± SE (N)	
	Dutch (NF54)	Honduras (H1)
Chloroquine	0.183 ± 0.040 (4)	0.037 ± 0.006 (6)
Mefloquine	0.022 ± 0.009 (6)	0.088 ± 0.006 (4)
Halofantrine	0.005 ± 0.002 (4)	0.009 ± 0.003 (6)
Qinghaosu	0.009 ± 0.002 (5)	0.028 ± 0.006 (3)
Amodiaquine	0.025 ± 0.007 (4)	0.032 ± 0.001 (4)
Quinine	0.13 ± 0.03 (4)	0.664 ± 0.02 (4)
Pyrimethamine	0.245 ± 0.06 (4)	3.40 ± 0.98 (6)
Sulfadoxine	117.1 ± 50.0 (4)	328.0 ± 201.5 (4)
Clindamycin	80.3 ± 34.5 (4)	264.0 ± 25.1 (4)
Erythromycin	58.8 ± 17.2 (4)	93.2 ± 50.0 (4)

(N = Number of experiment) ; (SE = Standard Error)

Table II
50% inhibitory concentration (IC50) of antimalarial drugs and antibiotics on 2 chloroquine-resistant strains of *Plasmodium falciparum in vitro*

Drug	Strain - IC50μm ± SE (N)	
	Tanzanian	Thai (K1)
Chloroquine	0.465 ± 0.09 (8)	0.534 ± 0.04 (6)
Mefloquine	0.060 ± 0.03 (8)	0.012 ± 0.003 (5)
Halofantrine	0.010 ± 0.002 (6)	0.004 ± 0.001 (4)
Qinghaosu	0.037 ± 0.002 (4)	0.014 ± 0.001 (4)
Amodiaquine	0.038 ± 0.003 (4)	0.018 ± 0.002 (6)
Quinine	0.423 ± 0.143 (8)	0.228 ± 0.035 (4)
Pyrimethamine	1.93 ± 1.02 (6)	47.8 ± 7.63 (6)
Sulfadoxine	266.0 ± 16.95 (4)	548.0 ± 151.5 (6)
Clindamycin	354.0 ± 166.0 (4)	288.0 ± 2.47 (4)
Erythromycin	156.0 ± 76.5 (4)	112.0 ± 6.7 (4)

(N = Number of experiment) ; (SE = Standard Error)

Table III
50% effective dose (ED50) of antimalarial drugs and antibiotics against the sensitive and resistant strains of *Plasmodium chabaudi* in mice

Drug	Effective dose (ED50%) $mgkg^{-1}$ ± SE	
	Sensitive strain (ES 317)	Resistant strain AS(3CQ)
Chloroquine	1.28 ± 0.21	2.03 ± 0.51
Mefloquine	2.34 ± 0.17	1.51 ± 0.05
Halofantrine	0.63 ± 0.05	0.17 ± 0.04
Amodiaquine	2.41 ± 0.12	2.42 ± 0.07
Quinine	38.48 ± 3.45	37.97 ± 1.35
Pyrimethamine	0.20 ± 0.001	0.47 ± 0.05
Sulfadoxine	0.05 ± 0.005	0.02 ± 0.001
Clindamycin	1.57 ± 0.02	1.32 ± 0.041
Erythromycin1	12.65 ± 11.21	148.20 ± 12.48

(SE = Standard Error)

B. Combination experiment

Table IV
Effect of individual *in vitro* combination studies

Drug combination	Parasite Strain	
	Sensitive Dutch (NF54)	Resistant Thai (K1)
Chloroquine - Quinine	ANTAGONISM	ADDITIVE
Clindamycin - Quinine	SYNERGISM	SYNERGISM
Pyrimethamine - Sulfadoxine	SYNERGISM	SYNERGISM
Chloroquine - Amodiaquine	ANTAGONISM	ANTAGONISM
Chloroquine - Erythromycin	SYNERGISM	SYNERGISM
Amodiaquine - Erythromycin	ADDITIVE	ADDITIVE
Amodiaquine - Clindamycin	ANTAGONISM	ADDITIVE

Table V
Effect of individual *in vitro* combination studies

Drug	Parasite Strain	
	Sensitive Dutch (NF54)	Resistant Thai (K1)
Chloroquine – Quinine	ADDITIVE	ANTAGONISM
Clindamycin – Quinine	ADDITIVE	SYNERGISM
Pyrimethamine – Sulfadoxine	SYNERGISM	SYNERGISM
Chloroquine – Amodiaquine	ANTAGONISM	ANTAGONISM
Chloroquine – Erythromycin	SYNERGISM	SYNERGISM
Amodiaquine – Erythromycin	ADDITIVE	ADDITIVE
Amodiaquine – Clindamycin	ANTAGONISM	ADDITIVE

Discussion

Single Drug Experiment

A. in vitro

The experiment confirms that H1 and NF54 were sensitive to chloroquine while K1 and Tanzanian were resistant. However, the difference in results between strains against chloroquine as indicated by IC50's of H1 (0.0365 μm) and NF54 (0.183 μm), as both were originally chloroquine sensitive, may be due to the number of passages *in vitro* undergone by the parasite line cultures at the time of experiment. Experiments on NF54 strain were carried out after more passages

than H1. Cultures which had been passaged *in vitro* for longer time were reported by Le Bras *et al*[28] as being more resistant to chloroquine. The sensitivity of the strains tested here to newer drugs such as mefloquine, qinghaosu and halofantrine support the potentials of these drugs for treatment of chloroquine-resistant malaria. Amodiaquine still remains the drug of choice for treatment of chloroquine-resistant malaria. Its IC50 values for all the strains could be considered equally effective as any of the newer drugs.

In this experiment the antibiotic clindamycin and erythromycin gave higher IC50 values than the other drugs which would appear to indicate their ineffective antimalarial activity. Clindamycin had a slow blood schizontocidal activity[29,30] and it has been demonstrated to have significant antiparasitic effects at concentrations near those observed during therapy *in vivo*[31].

Sulfadoxine was relatively ineffective against all the strains tested. The lower effect of the drug could be due to the medium used for the test[32] as RPMI medium contained as high concentration of p-aminobenzoic acid (PABA) that competed with the drug.

B. in vivo

The sensitivity of both strains of parasites to amodiaquine, quinine and clindamycin confirms the usefullness of these drugs in treating chloroquine-resistant strain. Also it shows that the two newer drugs, mefloquine and halofantrine, were very active on the resistant strain. The AS(3CQ) strain was resistant to pyrimethamine with an ED50 of 0.47mgkg^{-1}. Apparently this strain was made resistant to pyrimethamine before making it resistant to chloroquine. Both antibiotics, clindamycin and erythromycin, showed different effect. Clindamycin was more effective against the resistant parasites than erythromycin. Erythromycin seemed to be less effective against both strains and Warhurst *et al*[33] showed that erythromycin given alone was not very effective against *Plasmodium berghei* infection in mice. Unlike the *in vitro* studies, sulfadoxine was very effective against the resistant strain. Sulfadoxine interferes with the use of PABA by the parasites and resistance of parasites to these agents be due to an increased production of PABA by the parasites.

Combination Drug Experiment

A. In vitro

Therapy with combination of drugs is warranted for the avoidance of emergence of drug-resistant organism, and to lessen adverse reactions by using smaller doses of each individual drug. Tables I and II show that both pyrimethamine and sulfadoxine when used alone were not very effective but showed potentiative synergistic action when both of them were combined together *in vitro* (Table IV). Each drug seemed to stimulate the activity of the other. Synergism occured since one drug competed with PABA (sulfadoxine) and the other drug inhibited dihydrofolate acid reductase (pyrimethamine).

Strains NF54 and K1 of *Plasmodium falciparum* were less sensitive to clindamycin and erythromycin. The early interest in the antimalarial properties of antibiotic slowed down as the compounds were slow acting[31].

Erythromycin given alone is not very effective but in combination with chloroquine shows potentiative synergism with chloroquine against *Plasmodium berghei* - chloroquine resistant strains[33]. Later on, Warhurst[34] suggested the possible effect of chloroquine was to increase mitochondrial membrane permeability and increase its sensitivity to erythromycin. Gershon and Howells[35] showed the same results when chloroquine - erythromycin combination acted against *Plasmodium falciparum in vitro.*

Clindamycin itself is relatively ineffective against *Plasmodium falciparum* but the antibiotic showed potentiative synergism when combined with quinine. This was seen against the resistant strain K1 but effects against the sensitive strain (NF54) were additive (Table IV). Miller *et al*[29], Clyde *et al*[30] and Hall *et al*[36] found the use of quinine and clindamycin a suitable therapy for multiresistant *Plasmodium falciparum.*

The combination of chloroquine with other antimalarials such as quinine or amodiaquine was additive (Table IV) against NF54 or antagonistic for K1. Each drug seemed to lower the efficacy of the other. The suggestion that quinine following chloroquine -resistant strains showed up an antagonistic effect was made by Hall[37]. Unlike chloroquine,

amodiaquine failed to produce any synergistic effect when combined with erythromycin. Instead they were all additive as shown in Table IV for both NF54 and K1. The same result for K1 was obtained when amodiaquine was combined with clindamycin but antagonism was shown for NF54.

In vivo

Combination therapy may be used to obtain increased antimalarial activity as compared with a single drug. The combination of a dihydrofolate reductase (DHFR) inhibitor and a sulphonamide is an example. Both agents inhibit folic acid synthesis and when used together greatly enhanced the activity of either compound alone. This synergistic combination of sulfadoxine and pyrimethamine is the most widely used combination of a DHFR inhibitor and a sulphonamide.

Erythromycin alone was less effective but observed to potentiate the action of chloroquine against the two strains of parasite tested as seen in Table IV. Erythromycin is considered to act on bacterial and mitochondrial ribosomes[38]. The mechanism is thought to be due to the action of chloroquine on membrane[39] in particular on mitochondrial membrane. A raised chloroquine concentration inside the malaria parasite might increase the permeability of the mitochondrial membrane to erythromycin.

The combination of clindamycin-quinine showed a marked potentiation on both sensitive and resistant strains of *Plasmodium berghei* (Table IV). The possible explaination is that since clindamycin affects mitochondrial and bacterial ribosomes, perhaps quinine treatment increases the ability of the antibiotic to enter the mitochondrion, as suggested for chloroquine and erythromycin[34].

The antagonistic effect shown by chloroquine when combined with quinine on a sensitive strain was also demonstrated by several studies with *Plasmodium falciparum* in man[37,40] and Reed *et al*[41] showed that chloroquine had a dose-related inhibitory effect on the success rate when combined with quinine. They found that quinine alone had a success rate of 50% but the more the amount of chloroquine added to quinine the lesser the success rate obtained.

Antagonistic effect was observed when chloroquine combined with amodiaquine, amodiaquine-clindamycin. It seems that amodiaquine tended to lower the efficacy of the other drug perhaps because the drugs belonged to the same group (chloroquine and amodiaquine belong to 4-aminoquinolines) they may compete for uptake sites.

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References

1. WHO. The epidemiology of drug resistance of malaria parasites. Memorandum from a WHO Meeting. Bull World Health Organ 1987;65 : 797-816.

2. Payne D. The history and development of the WHO standard *in vivo* and *in vitro* test system for the sensitivity of *Plasmodium falciparum* and other human plasmodia to antimalarial drugs. Ph.D Thesis, University of London 1989.

3. Nahlen RL, Akintunde A, Alakija T *et al.* Lack of efficacy of pyrimethamine prophylaxis in pregnant Nigerian women. Lancet 1989;ii : 830-3.

4. Lemnge M and Inambao AW. *In vivo* and *in vitro* sensitivity of *Plasmodium falciparum* to chloroquine at Lubwe and Kalene in Zambia: Use of amodiaquine as an alternative antimalarial drug. Trans R Soc Trop Med Hyg 1988;82 : 194-6.

5. Kremsner PG, Zotter GM, Feldmeier H *et al. In vitro* drug sensitivity of *Plasmodium falciparum* in Acre, Brazil. Bull World Health Organ 1989;67 : 289-93.

6. Pinichpongse S, Doberstyn EB, Cullen JR, Yisunsri L, Thongsombun Y and Thimasam K. An evaluation of the five regimens for the out patient therapy of falciparum malaria in Thailand, 1980-1982. Bull World Health Organ 1982; 60: 907-12.

7. Qilin H, Weichuan O, Jiexian Z *et al.* Effectiveness of amodiaquine, sulfadoxine-pyrimethamine and combinations of these drugs for treating chloroquine resistant falciparum malaria in Hainan Island, China. Bull World Health Organ 1988;66 : 353-8.

8. Strickland GT, Khaliq AA, Sarwar M, Hassan H, Perveez M and Fox E. Effects of Fansidar on chloroquine resistant *Plasmodium falciparum* in Pakistan. Am J Trop Med Hyg 1986;35 : 61-5.

9. Maynadie M, Peceno C, Noriega PL and Yarzabal L. Susceptibility of *Plasmodium falciparum* strains to chloroquine and mefloquine in the Amazonas Federal Territory of Venezuela. Trans R Soc Trop Med Hyg 1989;83 : 586-8.

10. Wernsdorfer WH and Payne D. The dynamics of drug resistant in *Plasmodium. Falciparum* In: International Encyclopaedia of Pharmacology and Therapeutics 1990 (in press)

11. Isaacs RD and Ellis-Pegler RB. *Plasmodium falciparum* RI resistance to quinine and sulfadoxine-pyrimethamine in the Solomon Islands. Med J Aust 1987;146 : 449-50.

12. Bisseru B. Chloroquine-resistant *Plasmodium falciparum* in Zambia. Central Afr J Med 1988;34 : 17-8.

13. Nguyen C, Gregson D, Mouldey P and Keystone JS. Falciparum malaria resistant to to chloroquine, quinine and Fansidar in a non-immune patient infected in Ghana. Trans R SocTrop Med Hyg 1989;83 : 485-6.

14. Warhurst DC, Hall AP and Tjokrosonta S. RI quinine-Fansidar resistant falciparum malaria from Malawi. Lancet 1985;ii : 330.

15. Brandicourt O, Druilhe P, Diouf F, Turk P and Danis M. Decreased sensitivity to chloroquine and quinine of some *Plasmodium falciparum* strains from Senegal in September 1984 .Am J Trop Med Hyg 1986;35 : 717-21.

16. Brasseur P, Pathe-Diallo M and Duilhe P. Low sensitivity to chloroquine and quinine of *Plasmodium falciparum* isolates from Guinea in March, 1986. Am J Trop Med Hyg 1987;25 : 14-9.

17. Brasseur P, Koyamono J, Brandicourt O, Moyou-Somo R and Druilhe P. Patterns of *in vitro* resistance to chloroquine, quinine and mefloquine of *Plasmodium falciparum* in Cameroon, 1985-1986. Am J Trop Med Hyg 1988;39 : 166-72.

18. Nosten F, Ter Kuile FO, Chongsuphajaisiddhi T *et al.* Mefloquine resistant falciparum malaria on the Thai-Burma Border. Lancet 1991;i : 1140-3.

19. Ter Kuile FO, Dola G, Nosten F *et al.* Halofantrine versus mefloquine in the treatment of multidrug resistant falciparum malaria. Lancet 1993; 341: 1044-9.

20. Bygbjerg IC, Schapira A, Flachs H, Gomme G and Jenpsen S. Mefloquine resistance of falciparum malaria from Tanzania enhanced by treatment. Lancet 1983;i : 774-5.

21. Ringwald P, Bartczak S, Lew Bras J, Bricaire F, Matheron S and Coulaud JP. Antimalaria prophylaxis failure with mefloquine in Africa. Trans R Soc Trop Med Hyg 1990 (in press).

22. Ponnudurai T, Leeuwenberg ADEM and Weuwissen THETh. Chloroquine sensitivity of isolates of *Plasmodium falciparum* adapted to *in vitro* culture. Trop Geogr Med 1981;33 : 50-4

23. Nguyen-Dinh P, Hobbs JH and Campbell CC. Assessment of chloroquine sensitivity of *Plasmodium falciparum* in Choluteca, Honduras. Bull World Health Organ 1981;59(4) : 641-6

24. Irare SGM. Evaluation of the sensitivity and mode of action of three new antimalarial drugs. Ph.D Thesis, University of London 1983.

25. Thaithong S, Beale GH and Chumongkonkul M. Susceptibility of the *Plasmodium falciparum* to five drugs: an *in vitro* study of isolates mainly from Thailand. Trans R Soc Trop Med Hyg 1983; 77(2): 228-31.

26. Peters W. Chemotherapy and Drug Resistance in Malaria. London: Academic Press 1970.

27. Padua RA. Genetics of resistance to chloroquine. Exp Parasitol 1981;52(3) : 419-26.

28. Le Bras J, Deloron P, Ricour A, Andrieu B, Savel K and Coulaud JP. *Plasmodium falciparum*: Drug sensitivity *in vitro* of isolates before and after adaptation to continuous culture. Exp Parasitol 1983;56(1) : 9-14

29. Miller LH, Glew RH, Wyler DJ *et al.* Evaluation of clindamycin in combination with quinine against multidrug resistant strains of *Plasmodium falciparum.* Am J Trop Med Hyg 1974;23(4) : 565-9.

30. Clyde DF, Gilman RH and McCarthy VC. Antimalarial effects of clindamycin in man. Am J Trop Med Hyg 1975;24(2) : 369-70.

31. Geary TG and Jensen JB. Effect of antibiotics on *Plasmodium falciparum in vitro.* Am J Trop Med Hyg 1983;32(2) : 221-5

32. Brockelman CR, Tan-Ariya P. Plasmodium falciparum in continuous culture. A new medium for the *in vitro* test for sulfadoxine sensitivity. Bull World Health Organ 1982; 60(3): 423-6.

33. Warhurst DC, Robinson BL and Peters W. The chemotherapy of rodent malaria. XXIV. The blood schizontocidal action of erythromycin upon *Plasmodium berghei.* Ann Trop Med Parasitol 1976;70(3) : 253-8.

34. Warhurst DC. Chloroquine-erythromycin potentiation in *Plasmodium berghei.* Ann Trop Med Parasitol 1977;71(3) : 383.

35. Gershon PD and Howells RE. Combinations of the antibiotics erythromycin and tetracycline with three standard antimalarials against *Plasmodium falciparum in vitro* Ann Trop Med Parasitol 1984; 78(1):1-11.

36. Hall AP, Doberstyn EG, Nanakorn A and Sonkom P. Falciparum malaria semi- resistant to clindamycin. BMJ 1975; 2 : 12-4.

37. Hall AP. Quinine and chloroquine antagonism in falciparum malaria. Trans R Soc Trop Med Hyg 1973;67(3) : 425.

38. Gale EF, Cundliffe E, Reynolds PE, Richard MH and Waring MJ. Antibiotic inhibitors of ribosome function. In: The Molecular Basis of Antibiotic Action. A Wiley-Interscience Publication 1981 : 474-7.

39. Inglot AD and Wolna E. Reaction of non-steroidal anti-inflammatory drugs with the rythrocyte membrane. Biochem Pharmacol 1968; 17: 269-79.

40. Brooks MH, Kiel FW, Sheehy TW and Barry KG. Acute pulmonary oedema in falciparum malaria. New Engl J Med 1968;279(14) : 732-7.

41. Reed WP, Feinstein M and Steiger BW. Early experiences in the treatment of falciparum malaria from South East Asia. J A M A 1968;205(1) : 131-3.