IN VITRO SENSITIVITY OF *PLASMODIUM FALCIPARUM* ISOLATES FROM BURMA TO CHLOROQUINE QUININE AND MEFLOQUINE

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INTRODUCTION

Emergence and spread of multidrug resistance to *Plasmodium falciparum* in Southeast Asia has been documented (Harinasuta *et al.*, 1976). In Burma, resistance to chloroquine was first observed in 1969 (Htun Nyunt and Than Myint, 1971; Clyde *et al.*, 1972; Aung Than Batu *et al.*, 1975) and it is now widespread all over Burma (Franco Tin and Nyunt Hlaing, 1973). In 1981, a community survey revealed the emergence of sulphadoxine-pyrimethamine resistance in Burma (Franco Tin, 1981).

Resistance in hospitalized patients have also been reported (Khin Mg Mg Than, 1976; Maung Maung Wint *et al.*, 1983; Kyaw Win and Aye Aung, 1983), while some were drug failures, some were demonstrated to be true drug resistant (Chit Maung *et al.*, 1981). Reduced efficacy to quinine in hospitalized patients have been reported by clinicians and also demonstrated in apparently healthy patients with *Plasmodium falciparum* infection in whom blood levels of quinine were also measured (Myint Lwin and Myint Oo, 1985). Because of the interference by host factors in *in vivo* drug testing, and also to find out the relationship among the *in vitro* response of the drugs in Burmese patients, the *in vitro* drug sensitivity testing to chloroquine, quinine and mefloquine was undertaken. The results are reported herein.

MATERIALS AND METHODS

Patients presenting themselves for treatment of malaria to an out-patient clinic in Rangoon city and to the Tharrawaddy township hospital, 80 miles north of Rangoon were screened. Those with asexual *Plasmodium falciparum* only, having parasite count between (6,000/c.mm-40,000/c.mm) and with no history of taking antimalaria therapy during the last 14 days were initially accepted. They were tested for the presence of antimalaria drugs, quinine, chloroquine, amodiaquine, sulphonamides in urine, and all those positive were excluded. In this study 26 patients were selected and accepted, 25 males and 1 female between the ages of 18 to 59 years.

The method of Dill and Glasko was used to detect 4 aminoquinolines, Mayer-Tanret reagent for quinine and Lignin test for sulphonamides in urine as described (WHO, 1981). The *in vitro* micro test was done according to the method described by Rieckmann *et al.*, (1978) using pre-charged plates supplied by WHO. Effective dose (ED_{50}, ED_{70}) i.e. the dose effective in reducing schizont formation by 50%, 70% were calculated by log graph using the probit analysis.

In order to compare the efficacy of 3 drugs in individual isolates, drug resistant values were determined by adding the percentage schizont maturation rate for each concentration of the different drugs according to methods decribed (Knowles *et. al.*, 1984). Thereafter the drug resistant values of individual subject were ranked for each drug, the highest rank order being assigned to the isolate with the highest resistant value, 1. The rank orders for each drug were then compared by Spearman's rank sum correlation test.

RESULTS

The effect of varying concentrations of chloroquine, quinine and mefloquine on schizont maturation of 26 isolates from Burma are illustrated in Figs. 1, 2 and 3 respectively. The isolates have been grouped according to the drug level at which complete growth inhibition occurs. It has been shown in 1982, that 2 chloroquine sensitive isolates of *P. falciparum* from Burma as determined by the WHO *in vivo* test were completely inhibited at 5.7 p.mol/well using the *in vitro* micro test (Myint Lwin *et al.*, 1981).

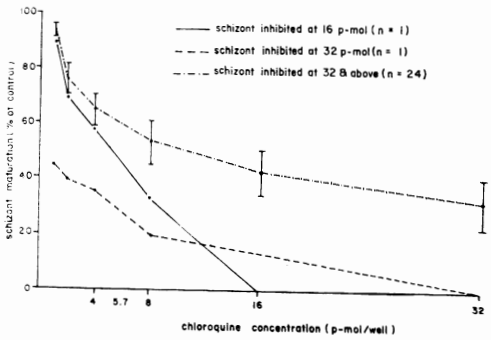

Fig. 1—Effect of chloroquine on maturation of *P. falciparum in vitro*.

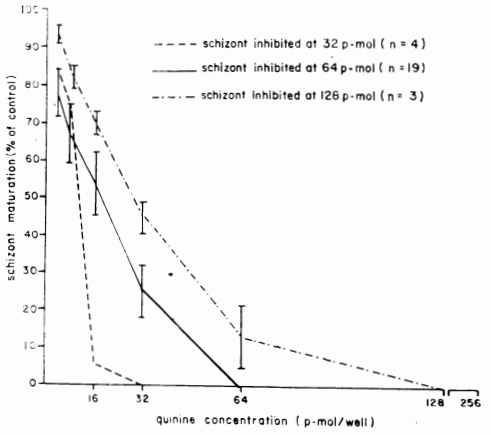

Fig. 2—Effect of quinine on maturation of *P. falciparum in vitro*.

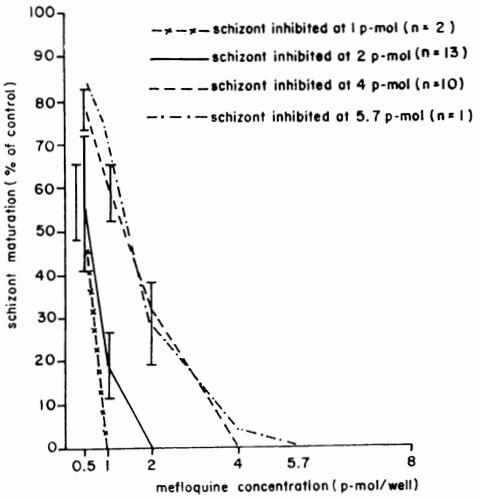

Fig. 3—Effect of mefloquine on maturation of *P. falciparum in vitro*.

The inhibition level in the present study far exceeds this value and all the isolates were highly resistant to chloroquine, 24 out of 26 were not inhibited at 32 p.mol, one was inhibited at 16 p.mol and the other at 32 p.mol.

The effective dose (ED_{50}, ED_{70}) of chloroquine on inhibition of schizont maturation *in vitro* has been calculated from the present series of 26 isolates and compared with ED_{50} and ED_{70} calculated from *in vitro* test which were performed in 1979 and 1981 on isolates obtained from similar case material, presenting for treatment to the same out-patient clinic in Rangoon. The 1979 data comprise 25 isolates, 23 resistant and 2 sensitive according to *in vivo* and *in vitro* tests (Ma Than Saw *et al.*, 1979). The 1981 data were from 45 isolates, on which the *in vitro* micro test was done and comprise 43 resistant and 2 sensitive patients according to *in vivo* test (Myint Lwin *et al.*, 1981).

Regarding quinine, 19 of the isolates were inhibited at 64 p.mol/well. It has been shown that maturation inhibition *in vitro* by quinine

concentration of 2.1-5.4 μg/ml of blood correlated with parasite clearance achieved with the administration of quinine sulphate 600 mg 8 hourly for 14 days (Sucharit *et al.*, 1970). According to this criteria, the maturation inhibition *in vitro* at 64 p.mol/well (4.1 μg./ml) in 19 of our isolates may be regarded as indicating sensitivity to quinine. In 3 of the isolates in which schizont maturation inhibition did not occur even at this level, but required a high level of 128 p.mol/well (8.1 μg. ml) may be taken as indicating resistant to quinine. The mean MIC of quinine calculated from the 26 isolates of this study was 4.3 μg/ml, ranging from 2.1 to 8.3 μg/ml.

The effect of mefloquine showed that 25 of the isolates were inhibited at less than 4 p.mol/well (0.3 μg/ml) and one isolate at 5.7 p.mol/well (0.4 μg/ml). The mean MIC of mefloquine on the 26 isolates in our study is 0.21 μg/ml ranging from 0.08 to 0.43 μg/ml.

Table 1

The drug resistant values and rank order for quinine, mefloquine and chloroquine in Burma.

Isolates	Drug resistant value (RV)			Rank order		
	Quinine	Mefloquine	Chloroquine	Quinine	Mefloquine	Chloroquine
KHM	322.3	186.2	330.8	1	2	20
KAT	316	153.6	399.3	2	7	16
KTT	284.2	77.8	301.4	3	19	21
KKL	277.1	159.5	515.2	4	3	2
KLK	271.7	154	558	5	6	1
DNM	263.1	142.8	432	6	9	11
KNT	258.1	98.8	406.5	7	13	15
KH	256.1	147.9	295.8	8	8	22
TTL	251.4	201.9	475.9	9	1	4
PKS	244	62.2	421	10	22	13
MTH	242.2	88.1	238.6	11	16	24
KSM	233.3	125.7	432.4	12	11	10
KZT	223.3	87.5	444.6	13	17	6
NMA	208	115.8	411.7	14	12	14
MMW	207.6	40.1	463.2	15	25	5
MKT	191.7	88.9	135	16	15	26
KNM	187.5	154.5	426.8	17	5	12
KMO	184.4	93	481.6	18	14	3
CHO	177.9	64.7	432.7	19	21	8
UKT	171.1	64.7	432.7	20	20	9
JHR	169.4	155.4	378.7	21	4	17
KMT	168.4	61.8	148.5	22	23	25
KTMC	165.7	137.5	361.6	23	10	18
KPL	165.7	34.1	288.4	24	26	23
KAM	164.6	79.3	437.3	25	18	7
KTA	139.1	42.9	330.9	26	24	19

RV = sum of percentage parasitaemia in six wells.
Rank order = rank number given according to resistant value; highest rank number = 1.

There was a significant correlation ($p<0.01$) between the MIC of quinine to mefloquine ($r = 0.48$). There was no correlation between MIC of other drugs.

The drug resistant ranked values of the isolates are shown in Table 1. Spearman's rank sum correlation test showed there was no correlation between the chloroquine and quinine ($rs = 0.13$) or chloroquine and mefloquine ($rs = 0.22$). However, the correlation was seen between mefloquine and quinine ($rs = 0.53$) with a significance at 1 % level.

Thorough history was taken from the 26 patients from whom the isolates were obtained. Except for 4 patients from Tharrawaddy, the history revealed that most of the patients were residents of Rangoon and had acquired malaria infections during the course of travel to different places in Burma, while some were resident elsewhere who had come to Rangoon for various reasons and presented themselves to the out-patient clinics.

DISCUSSION

The geographical spread of chloroquine resistance in Burma has been well documented. Since for practical therapeutic purposes it is the RII and RIII types of resistance that are important. Secular trends in the degree of resistance in Burma needs proper study. Definitive evidence for an increasing trend in degree of resistance requires repeated tests of a representative sample of the same population and this is not hitherto been possible in Burma. However many clinical reports indicated that proportion of RII and RIII resistant cases and degree of resistance to chloroquine is increasing. The increase in effective dose of chloroquine for 1979-1984 herein reported (Fig. 4) provides further confirmation of this increasing trend in degree of chloroquine resistance in Burma.

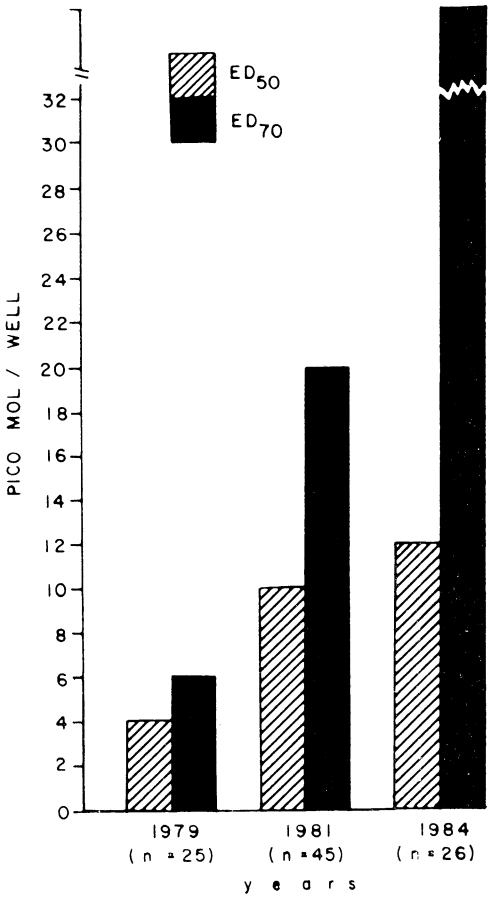

Fig. 4—Effective doses (ED_{50} & ED_{70}) of chloroquine in Burma.

Knowles *et al.*, 1984 using the cultured parasites and 5 drug concentrations in the *in vitro* macro test system, compared the rank order for drug resistant values between chloroquine, quinine and mefloquine and demonstrated a correlation between the response to chloroquine and quinine. We have used isolates from patients and 6 drug concentrations in the *in vitro* micro system and have not been able to obtain the correlation. We have not been able to include chloroquine sensitive strains in our study and may have missed any relationship that may really

exist. We have however been able to show a correlation between the rank order for drug resistant values of quinine and mefloquine.

The comparison of the MIC between the 3 drugs have indicated a correlation between quinine and mefloquine in our 26 isolates. However, the significance of this correlation is somewhat reduced because of the small numbers of isolates which were inhibited at 32 and 128 p.mol/well.

It has been demonstrated that *Plasmodium* species are capable of developing resistance to mefloquine under drug pressure *in vitro* (Brockelman *et al.*, 1981 and Smrkovski, 1982) and *in vivo* (Peters *et al.*, 1977). There is ample evidence that malaria parasites may develop cross resistance to drug with similar antimetabolic activity (Peters, 1970). The structural similarity between quinine and mefloquine (Howell, 1982) and relationship between resistance to quinine and mefloquine indicated in our present study suggest that mefloquine resistance may more easily develop in areas where there is already reduced sensitivity to quinine such as in Thailand and in Burma especially under increasing drug pressure.

SUMMARY

The *in vitro* sensitivity of 26 isolates of *Plasmodium falciparum* from Rangoon and Tharrawaddy areas in Burma were studied on chloroquine, mefloquine and quinine. The results indicated that the parasites were highly resistant to chloroquine but sensitive to mefloquine and quinine. The existence of correlation of sensitivity to mefloquine and quinine was detected and discussed.

No correlation between the parasite sensitivity to chloroquine and mefloquine and or chloroquine and quinine was detected.

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REFERENCES

AUNG-THAN-BATU, HTUN-NYUNT, R., NYUNT-HLAING, FRANCO-TIN, TIN-U, THAN-MYINT and KHIN-KYI-KYI, (1975). Chloroquine resistant malaria in Burma. *J. Trop. Med. Hyg.*, *78* : 186.

BROCKELMAN, C.R., MONGKOLKEHA, S. and TAN-ARIYA, P., (1981). Decrease in susceptibility of *P. falciparum* to mefloquine in continuous culture. *Bull. WHO.*, *59* : 249.

CHIT-MAUNG, MALAR-LWIN, SAW-PO-AUNG, SAW-HAN and S.J. THA, (1981). Sulphadimidine pharmacokinetics and its acetylator phenotyping in Burmese. Proceedings, Burma Medical Association, 76.

CLYDE, D. F., NYUNT-HLAING and FRANCO-TIN, (1972). Resistance to chloroquine of *P. falciparum* from Burma. *Trans. Roy. Soc. Trop. Med. Hyg.*, *66* : 369.

FRANCO-TIN, (1981). Alternative drugs to be used against multi-resistant *P. falciparum* malaria with special reference to drug combination comparative trials in Burma. *SEA/MAL/138.*

FRANCO-TIN and NYUNT-HLAING, (1973). Resistant to chloroquine of *P. falciparum* in Burma up to 1972. Burma Research Congress, Abstract, 96.

HARINASUTA, T., GILLES, H.M. and SANDOSHAM, A.A., (1976). Malaria in Southeast

Asia. *Southeast Asian J. Trop. Med. Pub. Hlth.*, *7* : 645.

HOWELLS, R.E., (1982). Advances in chemotherapy. *Brit. Med. Bull.*, 241.

HTUN-NYUNT, R. and THAN-MYINT, (1971). Chloroquine resistant malaria in Burma. First Report. Burma Research Congress, Abstract, 73.

KHIN-MAUNG-MAUNG-THAN, (1977). Comparison of sulfadoxine-pyrimethamine, sulfamethoxazole-trimethoprim and quinine in the treatment of chloroquine-resistant falciparum malaria. *Bur. Med. J.*, *23* : 17.

KNOWLES, G., DAVIDSON, W.L., JOLLEY, D. and ALPERS, M.P., (1984). The relationship between the *in vitro* response of *P. falciparum* to chloroquine, quinine and mefloquine. *Trans. Roy. Soc. Trop. Med. Hyg.*, *78* : 146.

KYAW-WIN and AYE-AUNG, (1983). Resistance of *P. falciparum* to sulphonamide compounds. *Bur. Med. J.*, *29* : 117.

MAUNG-MAUNG-WINT, MYO-THWE and TIN-NU-SWE, (1983). Alternative drug regimens for chloroquine resistant falciparum malaria. *Bur. Med. J. 29* : 101.

MYINT-LWIN, MIN-ZAW and ROONEY, W., (1981). Comparative study on the micro *in vitro* and the *in vivo* test on the response of *P. falciparum* to chloroquine in Burma. WHO Meeting on Drug Resistant Malaria. Kuala Lumpur, 10-15. August, 1981.

MYINT-LWIN and MYINT-OO, (1985). Study on oral quinine therapy in patients with falciparum malaria. *Bur. Med. J.*, *31* (S) : 4.

PETERS, W., (1970). Chemotherapy and Drug Resistance in Malaria. Academic press. London and New York.

PETERS, W., PORTUS, J. and ROBINSON, B.L., (1977). The chemotherapy of rodent malaria. XXVIII. The development of resistance to mefloquine (WR 142 490). *Ann. Trop. Med. Parasit.*, *71* : 419.

RIECKMANN, K.H., CAMPBELL, G.H., SAX, L.J. and MREMA, J.E., (1978). Drug sensitivity of *P. falciparum*. An *in vitro* micro technique. *Lancet*, *1* : 221.

SMRKOVSKI, L.L., ALCASTARAM, A., BUCK, R.L., ODRIGUEZ, C.S., UYLANGCO, C.B., (1982). *In vitro* mefloquine resistant *P. falciparum* from the Philippines. *Lancet*, *2* : 322,

SUCHARIT, P., SUNTHARASAMAI, P., CHINTANA, T. and HARINASUTA, T., (1979). *In vivo* and *in vitro* studies of quinine sensitivity of *P. falciparum* in Thailand. *Southeast Asian J. Trop. Med. Pub. Hlth.*, *10*:138.

THAN-SAW, MIN-ZAW, THAWKA-KYIN and MYINT-LWIN, (1979). Correlation study of chloroquine sensitivity malaria *in vitro* (macro), *in vivo* and *in vitro* micro test. *SEARO/WHO.*

WHO, (1981). Chemotherapy of malaria. *WHO. Monogr. Ser.*, No. 27.