Analysis of Pfdmr 1 gene in mefloquine-resistant Plasmodium falciparum

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SUMMARY
Drug resistance in Plasmodium falciparum is a serious problem in most endemic areas. Recent studies have suggested the potential involvement of genes in the MDR gene family in resistance to quinoline-containing compounds in P. falciparum. In our present studies, a molecular analysis of pfdmr 1 in isolate strain of P. falciparum, 523a R, from Japanese mefloquine-resistant patient was done to determine the reported association of pfdmr 1 intragenic alleles and mefloquine resistance, and to examine the antimalarial activities of several antimalarial agents against the P. falciparum strain. The antimalarial activities against the strain was decreased susceptibility to mefloquine, artemisinin and halofantrine, in contrast increased susceptibility to chloroquine. The DNA sequence analysis of pfdmr 1 gene in a strain reveled no association of intragenic alleles with mefloquine resistance. Furthermore, the overexpression of pfdmr1 mRNA have been observed and it is about 7.2 times higher than sensitive strain. Our data shows that overexpression of pfdmr1 gene may be associated in mefloquine-resistance mechanism.

INTRODUCTION
Malaria remains one of the major world public health problems. Drug resistant strains of Plasmodium falciparum are increasingly prevalent in most endemic areas complicating treatment, control and prophylaxis rendering even newer therapeutic modalities rapidly useless (1). Chloroquine resistance in P. falciparum involves a decrease in chloroquine concentration in the parasite, and the rate of chloroquine efflux has been shown to be 40- to 50-fold more than in sensitive isolates (2). Other studies have suggested that decreased influx of chloroquine in resistant parasites is responsible for the phenotype (3). Mefloquine is an important drug, but the resistant parasites are also appeared and now increasing. It also found that concomitant with the increase in chloroquine resistivity was a decrease in the level of mefloquine resistivity and with amplification of the pfdmr 1 gene (4, 5). In our present study, we have selected one line of P. falciparum for mefloquine resistance and showed that the strain has resulted in increased expression of pfdmr 1 mRNA level, a decrease in chloroquine resistance and cross-resistance to halofantrine and artemisinin.

MATERIALS AND METHODS
Parasites. An isolated strain P. falciparum 523a S (isolated from mefloquine-resistant patient) was continuously cultured under progressively increasing mefloquine pressure. After two years, we got resistant parasite (designated as 523a R strain), which can able to grow at the concentration of 2.5x10^7 M mefloquine. The original strain (523a S) was cultured without the drug.

Assay for Drug Sensitivity. Parasites at an initial parasitemia of 0.3% were grown at 3% hematocrit in erythrocytes for 72 hr with dilutions of the antimalarial agents (chloroquine, quinine, halofantrine and artemisinin) against these strains (523a R strain), which can able to grow at the concentration of 2.5x10^7 M mefloquine. The original strain (523a S) was cultured without the drug.

Sequence Analysis of pfdmr 1 Gene and RT-PCR of pfdmr 1 mRNA Level. All of the amino acid residues at 86, 1034, 1042 and 1246 positions of pfdmr1 gene in chromosome 5 of P. falciparum...
(523a S and 523a R) were analyzed using Dye Terminator method followed by particular domain amplified by PCR, and compared with previously reported amino acid residues which thought to involved mefloquine resistance (7, 8, 9). The analysis of pfmdr 1 mRNA expression level was determined by RT-PCR analysis (10).

RESULTS AND DISCUSSION
It is found that, there is no differences in amino acid sequences between mefloquine-sensitive (523a S) and mefloquine-resistant (523a R) strain, although resistant strain showed about 10 times higher IC₅₀ value than sensitive strain. Moreover, 523a R strain showed decreased sensitivity to Artemisinin and halofantrine and increased sensitivity to chloroquine. The overexpression of pfmdr1 mRNA have been observed and it is about 7.2 times higher than sensitive strain.
Our results show that the drug resistance is not related with amino acid sequence and with polymorphism of pfmdr1 gene. It is likely that overexpression of pfmdr1 gene may be associated in mefloquine-resistance mechanism.

ACKNOWLEDGEMENTS
This work was supported in part by Grants for Scientific Research (12307007 and 13672286) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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