Available online at www.sciencedirect.com
SCIENCE DIRECT
Acta Tropica 98 (2006) 137–144
ACTA TROPICA
www.elsevier.com/locate/actatropica

Role of *pfmdr1* mutations on chloroquine resistance in *Plasmodium falciparum* isolates with *pfcrt* K76T from Papua New Guinea

Toshihiro Mita[a,*], Akira Kaneko[a,b], Francis Hombhanje[c], Ilomo Hwaihwanje[d], Nobuyuki Takahashi[a], Hikota Osawa[a], Takahiro Tsukahara[a], Andrew Masta[c], Jeffery K. Lum[e], Takatoshi Kobayakawa[a], Takashi Ishizaki[f], Anders Björkman[b]

[a] *Department of International Affairs and Tropical Medicine, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan*
[b] *Malaria Research Laboratory, Karolinska University Hospital, Karolinska Institutet, Stockholm 171 76, Sweden*
[c] *School of Medicine and Health Sciences, University of Papua New Guinea, P.O. Box 5623, Boroko, Papua New Guinea*
[d] *Wewak General Hospital, Wewak, East Sepik Province, Papua New Guinea*
[e] *Department of Anthropology, Binghamton University, Binghamton, NY 13902-6000, USA*
[f] *Department of Clinical Pharmacology and Pharmacy, School of Pharmaceutical Science, Teikyo Heisei University, Uruido 2289-23, Ichigaya City, Chiba 290-0193, Japan*

Received 9 September 2005; received in revised form 21 March 2006; accepted 21 March 2006
Available online 18 April 2006

Abstract

The N86Y mutation in *pfmdr1* is reported to play an additional role for the chloroquine resistance in *Plasmodium falciparum* isolates. However, not much has been done to clarify whether this mutation augments the level of chloroquine resistance in the isolates harboring *pfcrt* K76T mutation. We compared the in vitro chloroquine efficacy between *pfcrt* K76T mutant parasites with or without N86Y mutation from Papua New Guinea. A total of 57 isolates (4% sensitive, 14% borderline, and 82% resistant) were successfully tested in vitro for chloroquine sensitivity. We found a slightly higher effective concentration of chloroquine needed to inhibit *P. falciparum* by 50% (mean $EC_{50} = 107$ nM) in isolates with the *pfcrt* K76T + *pfmdr1* N86Y than that in isolates with the *pfcrt* K76T + *pfmdr1* N86 ($EC_{50} = 88$ nM), but this difference was not statistically significant. A significant non-random association was observed between the *pfcrt* K76T and *pfmdr1* N86Y alleles. Our results suggest that the *pfmdr1* N86Y mutation plays a compensatory role to chloroquine-resistant isolates under a chloroquine pressure while it may also augment the level of chloroquine resistance in the K76T parasites to a small extent.

Keywords: *Plasmodium falciparum*; Chloroquine resistance; In vitro; *pfcrt*; *pfmdr1*

1. Introduction

The alarming increase of the *Plasmodium falciparum* resistance to commonly used antimalarial drugs represents a major public health threat (Bjorkman and Bhattarai, 2005). Chloroquine-resistant *P. falciparum* first emerged in the late 1950s in both Southeast Asia and South America and have since spread rapidly to the most endemic regions (Wellems and Plowe, 2001).

In 2000, the *P. falciparum* chloroquine-resistant transporter (*pfcrt*) gene was identified (Fidock et al., 2000). The resistance was associated with a reduced accumu-

* Corresponding author. Tel.: +81 3 5269 7422; fax: +81 3 5269 7422.
E-mail address: hiro-tm@research.twmu.ac.jp (T. Mita).

0001-706X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2006.03.002

lation of chloroquine in the parasite digestive vacuole (Saliba et al., 1998) but how the *pfcrt* gene exerts such an effect on the digestive vacuole is largely unclear. An acquired efflux system of chloroquine (Sanchez et al., 2005) and/or an increased acidity in the digestive vacuole (Bennett et al., 2004) is assumed to play a role for the reduced accumulation. A lysine to threonine change at position 76 (K76T) has been found in every in vitro chloroquine-resistant parasite from around the world (Fidock et al., 2000; Babiker et al., 2001; Wootton et al., 2002). Many studies have confirmed the presence of K76T mutations in the in vivo chloroquine resistance but this mutation was not the sole requirement (Babiker et al., 2001; Djimde et al., 2001a; Pillai et al., 2001; Jelinek et al., 2002; Vinayak et al., 2003), suggesting that host factors affect the clearance of chloroquine-resistant parasites (Djimde et al., 2001a,b).

Another extensively investigated gene is the *P. falciparum* multiple drug resistance 1 (*pfmdr1*), a homologue of the mammalian multiple drug resistance gene encoding a P-glycoprotein on the chromosome 5 of the *P. falciparum*. It is a typical member of the ATP-binding cassette transporter superfamily localized to the parasite vacuole, where it may regulate intracellular drug concentrations (Duraisingh and Cowman, 2005). Mutation was observed at the 86, 184, 1034, 1042, and 1246 positions, which were strongly linked to the chloroquine resistance in laboratory clones obtained from various regions (Foote et al., 1990). Several field studies indicated the positive association between the asparagine to tyrosine change at position 86 (N86Y) and the chloroquine resistance both in vitro (Basco et al., 1995; von Seidlein et al., 1997; Grobusch et al., 1998; Duraisingh et al., 2000a) and in vivo (Gomez-Saladin et al., 1999; Nagesha et al., 2001). However, other studies have cast doubts about this association (Pillai et al., 2001; Thomas et al., 2002).

Currently, *pfmdr1* mutations are said to assist the chloroquine-resistant parasites by augmenting the level of resistance. Studies have shown that an introduction of the wild allele at positions 1034, 1042, and 1246 in *pfmdr1* into the chloroquine-resistant 7G8 clone resulted in the reduction of the drug concentration needed to inhibit parasite growth by 50%, EC_{50}, from 389 nM to 204 nM (Reed et al., 2000). Nevertheless, the mutant allele at these three positions introduced into the chloroquine-sensitive D10 clone was unable to confer chloroquine resistance. For the N86Y mutation, however, this association has not been fully confirmed in both experimental and field works.

In Papua New Guinea, oral 4-aminoquinoline (chloroquine for adults and amodiaquine for children) has been used for many years as the first-line treatment for uncomplicated malaria. However, the therapeutic efficacy of chloroquine decreased from 50–70% in the 1980s to 20–30% in the 1990s (Muller et al., 2003). The Department of Health and Welfare of Papua New Guinea, therefore, added sulfadoxine/pyrimethamine (SP) to the 4-aminoquinolines as their treatment policy for uncomplicated malaria in 2000. In this study, we determined the *pfcrt* and *pfmdr1* polymorphisms and in vitro sensitivity to chloroquine in *P. falciparum* isolates from East Sepik Province, Papua New Guinea, to explore the roles of *pfmdr1* gene mutations in chloroquine resistance.

2. Materials and methods

2.1. Study site and patients

The study was conducted in November 2002 and 2003 in Wewak, the capital of East Sepik Province, located on the northeast coast of Papua New Guinea. Malaria is hyperendemic and transmitted mainly by the *Anopheles farauti* and *A. koliensis*. Febrile children (1–14 years old) attending the Wewak clinic were screened for *P. falciparum* parasitemia. Blood films were stained with 10% Giemsa and examined microscopically. Criteria for recruitment in this study were: (1) asexual parasitemia from 1000 μL^{-1} to 80,000 μL^{-1} of blood, (2) no intake of antimalarial drugs during the preceding 4 weeks, (3) informed consent from the patient or parent. The detected parasitemic cases were treated with a combination of amodiaquine + SP according to the official treatment policy.

2.2. In vitro study

The World Health Organization (WHO) in vitro micro test kit was used to assess the sensitivity of *P. falciparum* to chloroquine. Briefly, a finger-prick blood sample was collected using heparinized capillary tubes (75 μL) (Drummond Scientific Company, Broomall, USA) and added to a RPMI 1640 medium (plus HEPES, $NaHCO_3$, and gentamycin 50 mg/mL) in a blood–medium ratio of 1:9. The 50 μL blood–medium mixture was pipetted into each well of the pre-dosed culture plate and incubated at 37.5 °C for 24–36 h in a candle jar. The wells A–H were dosed with 0 (control), 1, 2, 4, 8, 16, 32, or 64 pmol/well of chloroquine. After incubation, thick smears were prepared and stained with Giemsa. The test was defined as valid if a growth of ≥20 schizonts per 200 asexual parasites was obtained in the control well. A complete inhibition of parasite growth in well D (4 pmol/well) was classified as sensitive, parasite growth in well E