INTRODUCTION

It is estimated that there are about 500 million clinical attacks of malaria every year in tropical regions of the world, with over one million deaths, especially in children in sub-Saharan Africa (Greenwood et al., 2005). Control of this disease is handicapped by the existence of resistance in the most virulent of the four species of human malaria parasites, Plasmodium falciparum, to nearly all antimalarial drugs currently used in chemotherapy. Thus there is a need to find new drugs and to understand the mechanisms of their actions, so that rationale changes can be made to their structures to circumvent resistance once it appears in the parasite.

Malaria parasites develop in the red blood cells by digesting host hemoglobin within the parasite acidic food vacuole (Sherman, 1979). The low pH of this organelle is maintained by the activity of a vacuolar (V) H⁺-ATPase pump (Saliba et al., 2003). V-type H⁺-ATPase is also found on the parasite plasma membrane (Saliba and Kirk, 1999) and it has recently been shown that the malaria parasite exports this enzyme to the host red blood cell plasma membrane (Marchesini et al., 2005). Thus we have examined the inhibitory effects on P. falciparum growth in culture of concanamycin A, a macrolide antibiotic inhibitor of vacuolar H⁺-ATPase derived from Streptomyces sp (Drose et al., 1993). As it is unlikely that any new antimalarial drug will be used clinically as a monotherapy, we also investigated the combination of concanamycin A and pyronaridine (2-methoxy-7-chloro-10[3',5'-bis(pyrrolidinyl-1-methyl)-4''hydroxyphenyl] amino-benzyl(b)-1,5-naphthyridine), a new highly active blood schizonticidal Mannich base antimalarial drug developed in China, effective in treating chloroquine-resistant malaria (Looareesuwan et al., 1996).

MATERIALS AND METHODS

Parasite culture

P. falciparum strain K1 (chloroquine-resistant) (Thaithong and Beale, 1981) was maintained in culture under Trager and Jensen ‘candle jar’ condition (Trager and Jensen, 1976).

Assessment of in vitro antimalarial activity

The protocol of the [³H]-hypoxanthine in-
corporation method has been described previously (Auparakkitanon et al, 2003). In brief, aliquots of 200 µl of the parasite cell suspension (1.0% parasitemia of early ring stage) were incubated with 25 µl of the drug-containing medium in 96-well microtiter plates under candle jar condition for 24 hours at 37°C prior to the addition of 25 µl of 0.5 µCi [³H]-hypoxanthine (specific activity, 28.0 Ci/mmol; Amersham, Little Chalfont, United Kingdom). After a further incubation for 18 hours, parasites were harvested from each well onto glass fiber filters, and lysed with distilled water. Radioactivity on the filter discs was determined in a liquid scintillation counter. The IC<sub>50</sub> values (50% inhibition of radioactivity incorporation compared to control) were obtained from drug dose-response curves.

### Determination of drug combination

IC<sub>50</sub> values of one drug in the presence of a series of fixed concentrations of the other drug were measured as described above. Results were expressed as the mean sums of the fractional inhibitory concentrations (FIC), defined as (IC<sub>50</sub> of concanamycin A in mixture/ IC<sub>50</sub> of concanamycin A alone) + (IC<sub>50</sub> of pyronaridine in mixture/ IC<sub>50</sub> of pyronaridine alone) for each fixed concentration. Three types of drug interaction were defined as follows: additive, sum of FIC =1; synergism, sum of FIC < 0.5; antagonism, sum of FIC > 4. Results were also plotted as an isobologram.

### RESULTS

Assessment of the inhibitory effects of concanamycin A and pyronaridine on growth of P. falciparum K1 strain in culture yielded IC<sub>50</sub> value of 0.2 nM and 3nM respectively. Fig 1 shows an isobologram obtained from a plot of FIC of concanamycin A versus FIC of pyronaridine in a drug combination study of their antimalarial activity in vitro, demonstrating an additive effect (sum of FIC in the range 1.15 to 1.65).

![Fig 1–Isobologram of concanamycin A and pyronaridine. The solid line indicates an isobole where the two drugs act additively. FIC: fractional inhibitory concentration.](image)

### DISCUSSION

Although it is not possible to determine from the current study which of the various P. falciparum V-type H<sup>+</sup>-ATPases is the target of concanamycin A, the very high sensitivity of P. falciparum to inhibition by this macrolide antibiotic suggests that it may be acting on the parasite-encoded enzyme located at the host red blood cell plasma membrane, which is believed to play a key role in maintaining intracellular pH of the infected erythrocyte (Marchesini et al, 2005). However, the other parasite V-type H<sup>+</sup>-ATPases cannot be ruled out as possible targets. Alkalization of isolated P. falciparum digestive food vacuole has been shown to be achieved with 75 nM concanamycin A (Saliba et al, 2003).

We have recently shown that in vitro pyronaridine binds hematin, inhibits β-hematin (hemozoin) formation and enhances hematin-induced lysis of red blood cells (Auparakkitanon et al, 2006). Although localization of pyronaridine within the parasite acidic food vacuole has yet to be shown, ultrastructural changes caused by this drug to food vacuoles in intra-erythrocytic forms of P. falciparum have been reported (Wu et al,
WHO is currently developing a combination of pyronaridine and artesunate for treatment of malaria (www.who.int/mediacentre/news/notes/np8/en/). Examples of the therapeutic use of combinations of antimalarial with an antibiotic include quinine-tetracycline or doxycycline, quinine-clindamycin and chlorproguanil-dapsone (Lapdap) (Rosenthal, 2001).

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REFERENCES


