RESEARCH NOTE

INTERACTIONS BETWEEN ANTIPLASMODIAL 3,6-DIAMINO-1’-DIMETHYL-9-ANILINOACRIDINE AND HEMATIN AND CONCANAMYCIN A

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Abstract. Antiplasmodial 9-anilinoacridine derivatives exert their effects either by inhibiting DNA topoisomerase (topo) II or by interfering with heme crystallization within the parasite acidic food vacuole. Previous studies have shown that analogs of 9-anilinoacridine containing 3,6-diamino substitutions (in the acridine ring) inhibit Plasmodium falciparum DNA topo II in situ, whereas those with a 3,6-diCl substitution act by inhibiting beta-hematin formation, a property also seen with 3,6-diamino-1’-dimethyl-9-anilinoacridine (DDAA). To understand this seemingly anomalous property of DDAA, studies of its interaction with hematin and localization within the parasite food vacuole were undertaken. A weak interaction with hematin was demonstrated spectroscopically. Antagonism of DDAA inhibition of Plasmodium falciparum growth in culture by concanamycin A, a macrolide antibiotic inhibitor of vacuolar H+-ATPase derived from Streptomyces sp, was equivocal.

INTRODUCTION

With over 500 million new cases of malaria annually, a million deaths from infection with Plasmodium falciparum, particularly in children in sub-Saharan Africa, and the likelihood of emergence of parasites resistant to all currently employed antimalarials (Greenwood et al, 2005), there is an urgent need to identify new antiplasmodial compounds with the potential of being developed into effective and affordable drugs. The effectiveness of quinoline and acridine compounds, such as chloroquine and quinacrine, is due to their abilities to inhibit the process of heme crystallization to produce hemozoin, which occurs within the malaria parasite acidic food vacuole as a means of detoxifying the membrane-lytic property of free heme released following digestion of host hemoglobin (Tilley et al, 2001).

A number of derivatives of 9-anilinoacridine have shown promising antiplasmodial activity in vitro (Gamage et al, 1994). These compounds act either by inhibiting malaria parasite DNA topoisomerase II or by binding with hematin similar to that of chloroquine, thereby compromising hemozoin biogenesis. Analogs of 9-anilinoacridine containing 3,6-diamino substitutions (in the acridine ring) with both 1’-electron donating and 1’-electron withdrawing groups (in the aniline ring) inhibit DNA
topoisomerase II in situ as evidenced by their ability to produce DNA-protein adducts (Auparakkitanon and Wilairat, 2000). However, when inhibition of β-hematin (structurally identical to hemozoin) formation in vitro was compared among the antiplasmodial 9-anilinoacridines, analogs with a 3,6-diCl substitution together with an electron-donating group in the 1-anilino position were better inhibitors than those with 3,6-diamino substitutions (Auparakkitanon et al, 2003).

The potent antiplasmodial activity of 3,6-diamino-1-dimethyl-9-anilinoacridine (DDAA) (IC₅₀ value of 34 nM in chloroquine-resistant P. falciparum K1 in culture) has been attributed to its ability to inhibit DNA topoisomerase II (Auparakkitanon and Wilairat, 2000), but, paradoxically, it can also inhibit β-hematin formation (with an IC₅₀ value similar to chloroquine) although showing poor binding with hematin (Auparakkitanon et al, 2003). To clarify this situation, binding studies of DDAA with hematin using both spectrophotometric and spectrofluorometric methods were conducted. Localization of DDAA within the malaria parasite acidic food vacuole was also evaluated by examining whether its antiplasmodial function could be antagonized in the presence of concanamycin A, a macrolide antibiotic inhibitor of vacuolar H⁺-ATPase derived from Streptomyces sp (Drose et al, 1993).

MATERIALS AND METHODS

Parasite culture and in vitro inhibitory activity assay

P. falciparum K1 strain was maintained under “candle jar” conditions described by Trager and Jensen (1976) and in vitro inhibition was assessed using the [³H]-hypoxanthine incorporation method as previously described (Auparakkitanon and Wilairat, 2006). The IC₅₀ values (50% inhibition of parasite incorporation of radioactivity) were obtained from dose-response curves. In drug combination studies, IC₅₀ values of one drug (DDAA) in the presence of a series of fixed concentrations of the other drug (concanamycin A) were measured. The fractional inhibitory concentrations (FICs), defined as IC₅₀ of a given compound in a mixture/IC₅₀ of that compound alone, for each fixed concentration were plotted as an isobologram. If the mean sums of FIC = 1, the drug combination is considered as being additive; if the sum of FIC < 0.5, there is synergism; and if the sum of FIC > 4, it is antagonistic.

DDAA-hematin interaction assay

Interaction between DDAA and hematin was performed employing a continuous variation technique (Job’s plot) as described previously (Auparakkitanon and Wilairat, 2006). In brief, solutions containing the following 14 DDAA:hematin (molar) combinations were prepared in 2 mM sodium phosphate, pH 6, containing 40% (v/v) dimethyl sulfoxide: 0:1, 1:9, 1:4, 3:7, 2:3, 1:1, 3:2, 5:3, 13:7, 27:13, 7:3, 4:1, 9:1, 1:0; the final combined concentration of hematin plus DDAA in the mixtures was 10 µM. Absorption spectra of hematin and fluorescence spectra of DDAA were recorded in a Shimadzu UV-250 IPC spectrophotometer between 240-700 nm at a speed of 0.5 nm/minute and in a Shimadzu RF-5310 PC spectrofluorometer between 420 and 700 nm at an excitation wavelength of 414 nm. The absorbance peak of hematin at 400 nm was chosen for use in the Job’s plot, and the difference between the measured absorbance and expected value based on concentration added (absorbance change x -1) was plotted against the DDAA:hematin molar ratio.

RESULTS

Employing changes in hematin absorbance at 400 nm, the Job’s plot of the interaction between DDAA and hematin shows weak interaction, as indicated by the broad shape of the curve, with a stoichiometry of...
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hematin: DDAA of 2:1 (Fig 1). Similar experiments conducted by following the emission fluorescence of DDAA at 480 nm failed to show any changes in the fluorescence intensity (data not shown).

If DDAA interferes with hemozoin formation in situ, it should accumulate in the acidic food vacuole of the parasite where the heme target is located and thus be antagonized by concanamycin A, an inhibitor of vacuolar H⁺-ATPase. The isobologram obtained from FIC measurements of DDAA and concanamycin A combinations shows an additive effect, or a very mild antagonism (indifference) at best (sum of FICs ranging from 1.09-1.41) (Fig 2).

DISCUSSION

Analogs of 9-anilinoacridine exert their antiplasmodial effects either by inhibiting parasite DNA topoisomerase II (Auparakkitanon and Wilairat, 2000) or by interfering with β-hematin formation (Auparakkitanon et al, 2003). Their target specificity is governed, in part, by the type of side chains at the 3,6-positions of the acridine ring: the amino group directs specificity to DNA topoisomerase II and chloro group to hematin.

However, 3,6-diamino-1'-dimethyl-9-anilinoacridine (DDAA) demonstrates the DNA topoisomerase II inhibitory property in situ (Auparakkitanon and Wilairat, 2000), but it is also able to inhibit in vitro β-hematin production as efficiently as chloroquine (Auparakkitanon et al, 2003). This study showed a weak interaction of DDAA with hematin using both absorbance and fluoroscopic spectroscopic approaches, and the demonstration of its location within the malaria parasite acidic food vacuole was equivocal. As a Mannich base with two amino moieties in the acridine ring (Gamage et al, 1994), DDAA has the requisite property permitting accumulation in an intracellular acidic compartment. The lack of an antagonistic effect by concanamycin A on DDAA-induced parasite growth inhibition may be due to the low concentrations of concanamycin A (IC₅₀ = 0.2 nM) used in the drug combination tests, as a 300-fold increase in concentration is needed to produce alka-linization of the P. falciparum food vacuole.
Inhibition of β-hematin formation needs not require binding to hematin, and thereby preventing its involvement in the crystallization process, but conceivably can be achieved by blocking crystal growth through binding to the growing crystal lattice face as has been recently demonstrated for the action of artemisinin, chloroquine, diethylaminolokoxyxanthones and quinine (Solomonov et al, 2007). The ability of antiplasmodial drugs to have more than one target should make them attractive for future drug development as this would delay the onset of parasite drug resistance, a strategy underpinning current combination drug therapies.

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REFERENCES


