ASSESSMENT OF COMBINED ANTIMALARIAL ACTIVITY OF α, β ARTEETHER AND PRIMAQUINE AGAINST *PLASMODIUM BERGHEI, IN VIVO*

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Summary

Parasitic diseases like malaria, leshmania and trypanosomiasis are the major infectious disease which affects more than 30% of the world population in general and developing countries in particular, causing mortality and morbidity. These diseases pose a major challenge to drug discovery and delivery scientist due to their intracellular and strewn location. The treatment with drugs that targets the Plasmodium has become unpaid in recent years, since parasites have become resistant to the drugs. Poor rate of discovery in antiparasitic segments, high cost of the drugs had warranted effective management and delivery of existing drugs.

Present study evaluates the antimalarial activity of α β-arteether and primaquine combination in comparison with their respective monotherapy in *P.berghei* infected mice. Animals were observed for mortality and parasitemia progression. α β-arteether monotherapy at all the tested doses resulted in the recrudescence, while primaquine monotherapy failed to show any antimalarial activity. Administration of α β-arteether and Primaquine combination helps to reduce the therapeutic dosage of artemisinin as an antimalarial in the mouse model. In vivo, 3 oral doses of primaquine following a single injection of α,β-arteether to *Plasmodium berghei*-infected mice are able to prevent recrudescence due to α,β-arteether monotherapy and ensure almost 100% survival of the animals.

Key words: α β-arteether, Primaquine, *P.berghei*, Malaria, Recrudescence

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Introduction

Parasitic disease like malaria is one of the major infectious disease that affects more than 500 million people of the world population in general and in developing countries in particular, causing mortality and morbidity [1,2]. This infectious disease is caused by single-celled Plasmodium protists, including *P. falciparum, P. vivax, P. malariae, and P. ovale*. Among these, *Plasomodium falciparum* is the deadliest causing cerebral malaria. *P.vivax* has been known to account for 70-80 million cases annually. The parasites are usually transmitted from infected to uninfected population via the bite of female Anopheles mosquitoes where, approximately about 60 species of them serves as vectors. The treatment with drugs that kill the Plasmodium has become unpaid in recent years since; the parasites have become resistant to the drugs. Poor rate of discovery in antiparasitic segments, high cost of the drugs warrants the effective management of existing drug [3].

The roll back programme of the World Health Organization (WHO) against malaria have received a setback in view of resistance development (4, 5) and this has led to a policy decision to use combination therapy with artemisinin or its derivatives as the principle component (6).

Artemisinin is a sesquiterpene endoperoxide, isolated from the plant *Artemisia annua.* The plant extract is being used as traditional medicine in China for the last several years (7). Artemisinin is very effective and has fewer side effects. However, outside of the China and a few other neighboring countries, it is being used as an emergency drug and there are concerns in introducing it as a front line drug (8, 9). First of all, it is expensive and has some lasting concerns about safety. There is no much enough artemisinin available to treat all the cases globally and especially in Africa. Although attempts are underway to cultivate the plant in many countries and to rise the funding through private sector, artemisinin monotherapy suffers from the problem of recrudescence. Wide and suboptimal use as front line drug can lead to the development of resistance. Therefore, WHO has favored the development of the drug combinations with artemisinin or its derivatives, so that possible development of resistance to individual components is delayed and recrudescence due to artemisinin monotherapy is prevented (4,5).

The only registered combination antimalarial with artemisinin recently made available is artemisinin-lumifantrine (Co-artem, Novartis International AG, Basel, Switzerland).

Even though, efforts are underway to bring down the cost to less than US $ 1.0 (10, 12) by developing co-formulations, Co-artem is expensive, costing around US $2.4 per adult course, compared to the traditional chloroquine/SP therapy costing US $ 0.1-0.2.

Curcumin isolated from the roots of turmeric (*Curcuma longa*) was used as a partner drug with α β-arteether that has antimalarial activity in both *P. falciparum* culture and mice infected with *P. berghei (13).*

Over the years, Primaquine is the only available 8-aminoquinoline a^timalaòhal used as a hypnozoitocidal drug against *Plasmodium vivax* malaria and as a gametocytocidal drug against *P. falciparum* malaria. The WHO has recommended for some areas that primaquine, in a single dose should be added for the treatment regimens for *falciparum* malaria to reduce the transmissibility of the infection (14, 15). Methemoglobin toxicity and hemolytic anemia [deficient in the enzyme glucose-6-phosphate dehydrogenase (G6PD)] are common toxic effects associated with the primaquine (14, 15). Generally primaquine is prescribed for 14 Days. Due to longer duration of treatment patient may not complete the course once the symptoms of malaria are diminished. A shorter duration of chloroquine-primaquine treatment regimen was also tried out wherein primaquine was administered only for 5 days following the chloroquine course. However, the outcome of the treatment was not encouraging, since the percentage relapse was more than the standard 14 days primaquine treatment regimen (16).

The aim of the present study was to assess the value of adding primaquine to α β-arteether to overcome the problem of recrudescence at lowest possible concentration of both drugs.

Materials and Methods

Drugs and Chemicals

α β-arteether (a synthetic derivative of artemisinin) was a kind gift from IPCA Laboratories Ltd. (Mumbai India). Primaquine was a kind gift of IPCA laboratories Ltd. (Mumbai, India).

Pharmacodynamic evaluation: *in vivo* antimalarial efficacy testing in *P. bergheii* infected mice

In vivo antimalarial activity of (a) intramuscularly injected α β-arteether and orally fed primaquine as a single drug and (b) combination of intramuscularly administered α β-arteether and orally administered primaquine were evaluated.

The study protocol was approved by the Kuvempu University Ethical Committee. *P. bergheii* infected mice brought from the Indian Institute of Science, Bangalore was used as source and maintained in Albino mice (25±3 gm) through blood transfusion in fresh animal for eveòx five days. Blood was freshly withdrawn through heart puncture of infected mice (parasitemia >70%) and 100µl was injected intrapeòhtonially to the test animals. Animals were divided into 12-treatment groups of 6 animals each. The mice were maintained according to the endorsed procedure.

The animals were divided into two sets with each set having six groups (n = 6). In Set-I, the animals were divided as per the treatment mentioned. Group I (positive control, no drug treatment), Group II (1.5 mg /kg body wt primaquine), Group III (500 µgm α β-arteether /mice), Group IV (750 µgm α β-arteether /mice) and Group V (1.5 mg α β-arteether /mice) were tested individually for their antimalarial activity. In Set-II animals, antimalarial activity of combination of intramuscularly administered α β-arteether and orally administered primaquine was tested. Group VI (500 µgm α β-arteether /mice +1.5 mg /kg body wt primaquine), Group VII (750 µgm α β-arteether / mice +1.5 mg /kg body wt primaquine) and Group VIII (1.5 mg α β-arteether /mice +1.5 mg /kg body wt primaquine) were tested in combination for their antimalarial activity. Parasitemia level on the day of treatment was maintained to a minimum between 1-3 %. Animals were kept under observation to record the parasitemia and mortality. Blood smears were observed under microscope (100 X) to count parasites per 1000 RBC's per slide. These observations were recorded daily until all the animals from control group were died of malaria and then weekly for next 45 days for comparison.

Results

Striking results were obtained in terms of parasite clearance and protection against mortality. The experiments were carried out with 6 mice per batch and repeated at least 3 times.

The results presented in Fig.1 and Fig.2 indicates that α,β-arteether monotherapy at the indicated doses prolongs the survival of *P. berghei*-infected mice, but does not confer complete protection. Thus, while the *P. berghei*-infected positive control mice and primaquine treated mice (1.5 mg/ kg body wt primaquine) die between 5 to 8 days, a single injection of α β-arteether at 500µg, 750µg, and 1.5mg results in the death of animals between 11 to17, 23 to 31

and 28 to 38 days, respectively. However, treatment of the infected mice with α,β-arteether and primaquine results in better survival rates, and a 3-day oral regimen of primaquine with a single injection of α,β-arteether at 750µg or 1.5 mg per infected mouse led to complete protection of animals against recrudescence and 100% survival. The animals continued to enjoy normal health over two months as negative control animals. These observations were also supported by parasitemia index in the blood (Fig. 2). A single injection of α β-arteether was able to clear the parasites in blood, initially. But there was recrudescence and build up of parasitemia lead to the death of animals at various time intervals. The effective combination therapeutic doses gave complete clearance of parasitemia correlating with protection.

FIG. 1. Effect of Primaquine and arteether on mortality of *P. berghei* infected mice

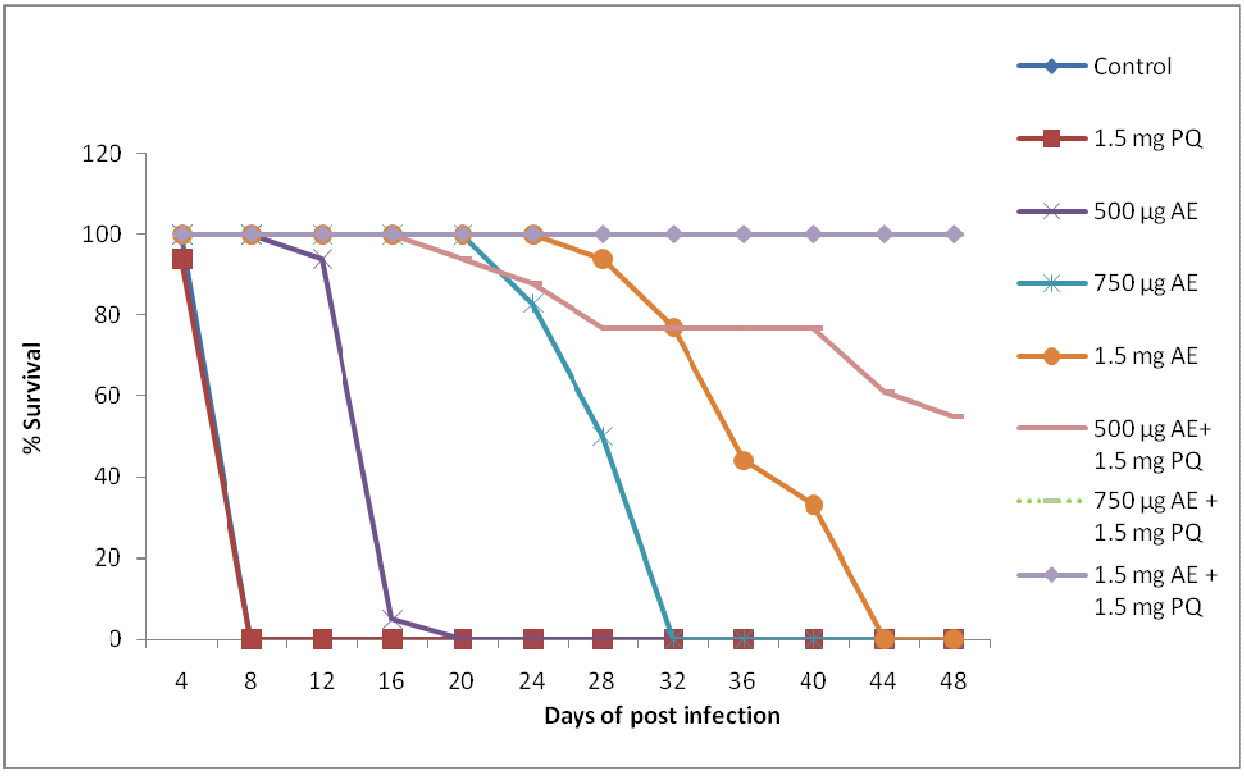
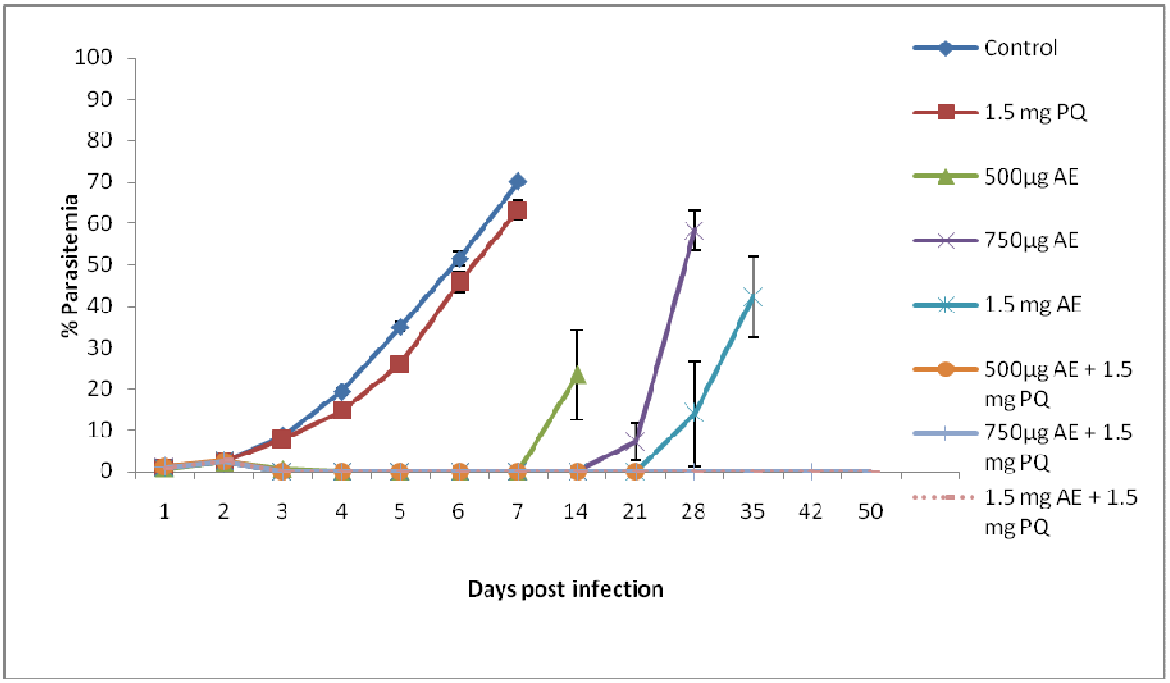


FIG. 2. Effect of Primaquine and arteether on parasite progression of *P. berghei* infected mice



Discussion

Primaquine is still widely used to treat *P. vivax* infections (17). It is believed that primaquine metabolically gets converted into an active quinine metabolite in the liver and interfere with parasite mitochondrial function [18]. Artemisinin derivatives are the most active of the antimalarial drugs against the asexual blood stages of malaria parasites. They also reduce gametocyte carriage in *P. falciparum* infections (19, 20). In earlier studies, primaquine in combination with quinine or artesunate had no additional significant effects on the activity of either drug against asexual blood stages in the human (11, 12). However, it is important to note that at these regimens drugs were not co-administered. But our data indicates that primaquine helps to reduce the concentration of α β-arteether against *P. bergheii* infected mice. It also observed that α ß- arteether and primaquine would need to be administered in tandem to achieve maximal beneficial effect as shown by earlier results for chloroquine and primaquine combination (23). α β- arteether, with its quick onset of action, will rapidly clear the parasite in

the erythrocytic stage, whilst the primaquine will help to clear the parasite in exo-erythrocytic stage thus preventing its development into erythrocytic stage. In case of α β- arteether monotherapy, with its quick onset of action it will rapidly clear the parasite in the erythrocytic stage but there will be no drug to take care of the exo-erythrocytic stage parasite which later develop into erythrocytic stage and results in the recrudescence (Fig. 1 and 2).

We suggest that the main advantages of α β-arteether / primaquine combinations compared with other possible combinations are; (a) complete cure without recrudescence at low cost, which is the main cause of concern in artemisinin based drug therapy. Extrapolating the effectiveness of the combination therapy in mice at 750 µg α β-arteether (single intramuscular dose) and 4.5 mg of primaquine (a total of 3 doses), the corresponding human dose would work out to 150 mg α β-arteether /70kg and 1.5 g/70 kg of primaquine (split into 3 doses). WHO has recommended a uniform 6 dose regimen of coartem against malaria in semi immune and non-immune patients. This works out to 480 mg artemisinin and 2850 mg of lumafentrine *over* a 3 day course of treatment. Artemisinin given as monotherapy needs a 7 day treatment at even higher doses and there is already deep concern of self medication at lower doses with potential for recrudescence and development of resistance in a few countries (5); (b) An α β-arteether / primaquine combination can lead to lower consumption of primaquine and decrease in the toxicity of drug (24). Our findings suggest that a novel formulation of two antimalarial drugs that are already licensed for use in humans could be used to treat chloroquine and artemisinin resistant parasites, thereby providing a cheap and effective means of extending the clinical life of α β-arteether. Clearly trials of optimized combinations of these drugs in humans are needed to determine whether any beneficial effect can be observed in field situations.

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