IN VIVO ANTIMALARIAL ACTIVITY OF ORAL BETA-ARTEETHER AND PIPERAQUINE COMBINATION.

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Summary

Malaria causes more than 3000 deaths each day world wide and is becoming more difficult to treat due to increasing drug resistance. Artemisinin based combinations has been advocated as the therapy of choice to handle wide-spread drug resistance in *Plasmodium falciparum* malaria. Present study evaluates the antimalarial activity of oral beta-arteether (AE) and piperaquine (PQ) combination in comparison with their respective monotherapy in *P. berghei* infected mice. Survival of the animals at the end of study period was taken as end point. Animals were observed on day 3, 5, 6, 9, 17 and 40 for mortality. Administration of beta-arteether and piperaquine combination resulted in survival of 80% animals unlike beta-arteether alone (40%) or piperaquine alone (40%) at the end of the study. Thus combination of oral beta-arteether and piperaquine may represent a potential therapeutic alternative to combat malaria infection.

Key words: Beta-arteether, Piperaquine, Plasmodium, *P.berghei*, Malaria

Introduction

Malaria is an infectious disease caused by the parasite plasmodia. *Plasmodium falciparum* and *Plasmodium vivax* are dominant pathogens of malaria that causes 300 million to 500 million clinical cases and more than 3000 deaths each day world wide (1).

Multidrug resistance has been reported from most parts of the world and as a result, monotherapy or some of the available combination chemotherapies for malaria are either ineffective or less effective. New antimalarial regimens are, therefore, urgently needed and antimalarial combination chemotherapy is widely advocated. Antimalarial combinations can increase efficacy, shorten duration of treatment (and hence increase compliance), and decrease the risk of resistant parasites arising through mutation during therapy (2,3,4).

Over the past decade, a new group of antimalarials – the artemisinin compounds, especially artesunate, artemether, arteether and dihydroartemisinin – have been deployed on an increasingly large scale. These compounds produce a very rapid therapeutic response (5). Artemisinin based combination therapy (ACT) has been advocated as the therapy of choice to handle wide-spread drug resistance in *Plasmodium falciparum* malaria, at the same time preventing recrudescence due to artemisinin monotherapy (6). WHO in its new Malaria guidelines (2006) recommends artemisinin combination therapy for uncomplicated falciparum malaria (7). Artemether/lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine, artesunate plus sulfadoxine/pyrimethamine are the combinations recommended by WHO.

A general issue with many of these combinations is side effects and pharmacokinetic mismatch between artemisinin and the other drugs in the combination. Another major concern of all these combinations is their high cost (8).Use of amodiaquine has been limited by its toxic side-effects like hepatotoxicity (9) and agranulocytosis (10). Both amodiaquine and sulfadoxine/pyrimethamine have high levels of pre-existing resistance. A disadvantage of artemether-lumefantrine is poor absorption of lumefantrine that is highly dependent on food intake, especially high fat food (11).

Beta-arteether (artemotil) is the beta-ethyl ether derivative of artemisinin. It is a blood schizonticide active against all stages of *P. falciparum*, including the very early ring forms. It is an effective and rapidly acting drug for the treatment of falciparum malaria. No serious side effects have been reported with beta-arteether. It was chosen by the Steering Committee of the Scientific Working Group on Malaria Chemotherapy of the WHO (CHEMAL) for further development. It has longer $t_{1/2}$ and more lipophilic properties than artemether (12). Being a lipophilic drug; it has better penetration as compared to other derivatives of artemisinin such as artesunate and artemether. Other advantages of beta-arteether are its stability, compared with sodium artesunate; its biochemical breakdown does not give methanol unlike artemether (13).

At present, beta-arteether is available only in injectable formulation and hence its combination with other antimalarial drugs is difficult. An oral form of beta-arteether was therefore developed so that it can be combined with long acting antimalarial.

Piperaquine is a bisquinoline antimalarial drug. The pharmacokinetic properties of piperaquine have also been characterized recently, revealing that it is a highly lipid-soluble drug with a large volume of distribution at steady state, long elimination half-life. The tolerability, efficacy, pharmacokinetic profile and low cost of piperaquine make it a promising partner drug for use as part of an ACT (14).

Fixed dose combination of beta-arteether with piperaquine will be effective combination therapy for malaria. Beta-arteether, with its quick onset of action, will rapidly reduce parasite biomass, whilst the long-acting activity of piperaquine is thought to prevent recrudescence. In the light of the above data, the present study was conducted to evaluate the antimalarial efficacy of a combination of oral beta-arteether and piperaquine to cure *P. berghei* infected mice and offer protection against recrudescence as compared to beta-arteether alone and piperaquine.

Material and Methods

Drugs and Chemicals

Beta-arteether (AE), Piperaquine (PQ) (both procured from Ipca Laboratories Ltd., Mumbai). These compounds were dissolved in dimethyl sulfoxide (DMSO) and stored at - 20^{o}C.

Experimental animals

Swiss male mice weighing 28-30g were obtained from the central animal facility, Indian Institute of Science, Bangalore, India. These animals were housed in 37cm × 23cm × 16cm polypropylene cages and acclimatized for a period of 7 days. Individual animals were identified by a mark on tail with permanent marker and cages were identified with label pasted on cage with relevant information. Animals were housed at a temperature of 24 $\pm$ 2 ^{o}C and relative humidity of 30 to 70 %. A 12:12 light: dark cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet. The experimental protocols were approved by the Institutional Animal Ethics Committee of the Institute.

Preparation and administration of doses

Study compounds Beta-arteether, Piperaquine were dissolved in Dimethylsulfoxide (DMSO) and the required concentration was administered in volume of 20 µl.

Induction of experimental malaria

P. berghei was maintained in Swiss mice obtained from the central animal facility, Indian Institute of Science, Bangalore, India. Blood obtained from mice with 60-70% parasitemia was diluted 3 times in Phosphate Buffered Saline (PBS) so that the concentration was 10^6 *P. berghei* infected erythrocytes per inoculum (0.1 ml). 0.1ml was injected intraperitonially into fresh mice for parasite propagation.

Experimental design

Twenty five Swiss mice were divided in to four groups of five animals each viz.. Group I (infected animals as control), group II (AE 1.5mg/mouse/day), group III (PQ 5mg/mouse, 3 doses/day) and group IV (AE 1.5mg + PQ 5mg/mouse/day).Animals in all the groups were inoculated with the parasitized blood on day 0. After 72 hours of infection (4 to 6% parasitemia), the study drugs were

administered orally to each group in different combinations as described above. The control group received vehicle DMSO orally. The animals in treatment groups received 3 doses of study drugs at 24 hours interval. Survival of the animals at the end of study period was taken as end point. Animals were observed on day 3, 5, 6, 9, 17 and 40 for mortality.

Results

All the animals in control group (Group I) died by day 6 due to parasitemia. The administration of beta-arteether (1.5mg/day) alone (Group II) or piperaquine (5mg/day) alone (Group III) resulted in only 40% survival at the end of the study. Administration of 3 doses at 24 hr interval of 1.5 mg beta-arteether and 5 mg piperaquine combination (Group IV) resulted in survival of 80% animals at the end of 40 day study. The results are given in Fig.1

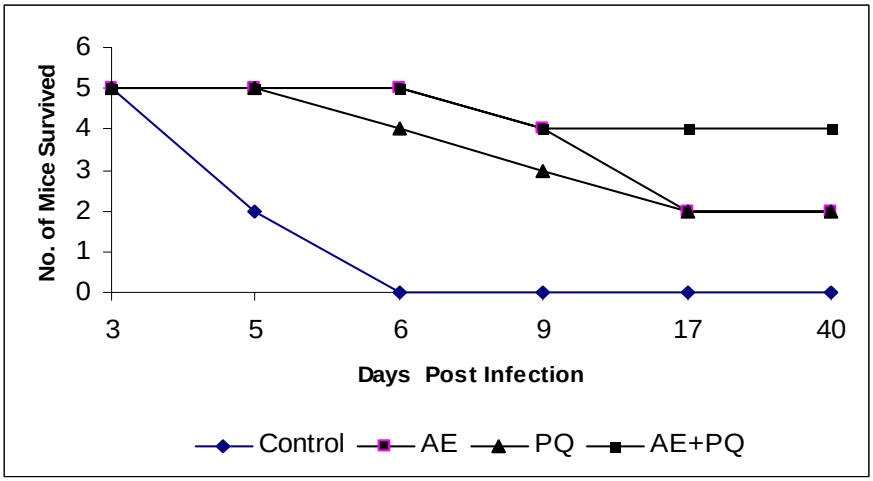

Fig. 1: Post treatment survival of animals in study treatment groups: Oral administration of combination of beta-arteether and piperaquine increases the survival of mice infected intraperitoneally with *P.berghei*. Three days after *P.berghei* infection mice were orally administered beta-arteether (1.5mg), piperaquine (5mg), combination of beta-arteether 1.5mg and piperaquine 5mg or vehicle as control once daily for a period of 3 days.

Discussion

Despite decades of intense research, malaria remains a deadly worldwide disease. Drug resistance to limited available antimalarials, in part, has contributed to the persistence of this infectious disease (15).

Piperaquine is a potent antimalarial agent and has been now regarded as an important partner in antimalarial treatment strategies, especially in combination with artemisinin drugs. It has been successfully combined with dihydroartemisinin or artemisinin with several advantages over established combinations such as artesunate -mefloquine and artemether-lumefantrine with respect to greater efficacy, improved adherence, better tolerability and lower cost (16).

Beta arteether a rapidly acting artemisinin derivative is conventionally available only in injectable form that makes the combination of beta-arteether with piperaquine difficult. In this study a novel oral fixed dose combination of beta- arteether with piperaquine was tested for its antimalarial activity in *P.berghei* infected mice. This is probably the first study to demonstrate in-vivo antimalarial activity of this combination.

Although primate models provide a better prediction of efficacy in humans than the rodent models, the latter have also been validated through the identification of several conventional antimalarials such as chloroquine, halofantrine, mefloquine and more recently artemisinin derivatives (2).

The antimalarial activity of oral beta-arteether and piperaquine combination was compared with antimalarial activity of individual components of this combination monotherapy (beta-arteether alone and piperaquine alone).

The study demonstrated that both beta-arteether alone at 1.5mg/mouse (3 doses) and piperaquine alone at 5mg/mouse (3 doses) is partially protective (only 40% mice survived at the end of the study). The combination of beta-arteether with piperaquine (3 doses) gave 80% protection.

These results indicate that though beta-arteether and piperaquine monotherapy at the indicated doses, exhibit antimalarial activity and prolong the survival of *P. berghei* infected mice, it does not confer complete protection (only 2 mice out of 5 mice survived at Day 40). Combination of beta-arteether with piperaquine results in better survival rate (4 out of 5 mice survived at Day 40) and hence is a better alternative as compared to monotherapy. Combination of beta-arteether and piperaquine showed synergistic antimalarial activity. Being a preliminary evaluation of antimalarial activity of this combination parasitemia suppressive test was not performed.

Combination of oral beta-arteether and piperaquine is superior as compared to its individual components in terms of survival rates. The combination can provide a potential therapeutic alternative to combat drugs resistant falciparum malaria. Further preclinical studies evaluating effect on parasitemia and well controlled clinical studies are required to explore the therapeutic potential of this combination in patients with falciparum malaria.

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