ASSESSMENT OF CURCUMIN-PRIMAQUINE COMBINATION THERAPY FOR MALARIA

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Summary

The existing armamentarium of drugs for the treatment and prevention of malaria is inadequate due to development of resistance. However, most of these drugs still have a place and their life-span could be prolonged if better deployed and used, and also by rationally combining them based on pharmacodynamic and pharmacokinetic properties. Present study evaluates the antimalarial activity of curcuminoids and primaquine combination in comparison with their respective monotherapy in *P.berghei*-infected mice. Animals were observed for mortality for a period of one month. Curcumin helps to reduce the therapeutic dosage of primaquine. *In vivo*, five oral doses of primaquine following five oral doses of curcumin to *Plasmodium berghei*-infected mice are able to prevent recrudescence due to primaquine monotherapy and ensure 50% survival of the animals for more than 30 days.

Key words: Resistance, Combination therapy, Recrudescence, Oral dose, Immunomodulatoin

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Introduction

The existing armamentarium of drugs for the treatment and prevention of malaria is inadequate due to development of resistance. Newer compounds are also being developed, but unfortunately due to lack of incentives in the antiparasitic drug discovery area resulted in only few newer compounds being introduced in to the market since several years which is insufficient to tackle the problem (1). However, most of older drugs still have a place and their life-span could be prolonged if better deployed and used, and also by rationally combining them based on pharmacodynamic and pharmacokinetic properties. The nature of malaria disease and its prevalence in the developing world call for innovative approaches to develop new affordable drugs and to safeguard the available ones. According to WHO, the concept of combination therapy is based on the synergistic or additive potential of two or more drugs, to improve therapeutic efficacy and also delay the development of resistance to the individual components of the combination. Combination therapy (CT) with antimalarial drugs is the simultaneous use of two or more drugs with independent modes of action and different biochemical targets in the parasite.
Over the years, primaquine is the only available 8-aminoquinoline antimalarial used as a hypnozoitocidal drug against *P. 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 (2, 3). Generally primaquine is prescribed for 14 days. However, methemoglobin toxicity and hemolytic anemia [in patients deficient in the enzyme glucose-6-phosphate dehydrogenase (G6PD)] are common toxic effects associated with the primaquine (2, 3). Due to longer duration of treatment patients may not complete the course once the symptoms of malaria are diminished. A shorter duration of chloroquine-primaquine treatment regimen was also tried out where in 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 (4). Curcumin isolated from the roots of the *Curcuma longa* plant has been shown to regulate a number of biological responses (5). Recently, curcumin was reported to posses a prominent antimalarial activity both *in vitro* (on chloroquine resistance and sensitive strains) and *in vivo* (on *P. berghei*) studies (6, 7). Due to its relative abundance and cost effectiveness this therapeutic molecule may be more suitable to developing countries which evidence the maximum malaria cases. The aim of the present study was to evaluate the therapeutic benefit of curcumin-primaquine combination therapy for malaria in *P. berghei*-infected mice model.

Materials and Methods

Curcumin was purchased from HI MEDIA (Mumbai, India). Primaquine was a kind gift of IPCA laboratories Ltd. (Mumbai, India). Field Stain A and B (HI MEDIA, Mumbai, India), Methanol (HPLC grade, HI MEDIA, Mumbai, India). All other reagents and chemicals obtained are of analytical grade.

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

In vivo antimalarial activity of (a) orally administered curcumin (b) combination of orally administered curcumin and primaquine combinations was evaluated. The animal study protocol was approved by the Kuvempu University Animal Ethical Committee. Animal experiments were carried out according to the CPCSEA (Committee for the purpose of the control and supervision on experiments on animals) guidelines. *P. berghei*-infected mice brought from the Indian Institute of Science, Bangalore. Swiss mice (25 to 28 g) were injected intraperitoneally with *P. berghei*-infected mouse blood (70 to 80% parasitemia) on day 0, such that the animals developed high parasitemia and died in about 5 to 8 days. Animals were divided into 6 treatment groups of 6 animals each. The mice were maintained according to the endorsed procedure.

The animals were divided into six groups with each group having six groups (n = 6). After four hours of infection, the animals were treated with the primaquine drug solution (PQDS) and curcumin drug solution (CUDS)**.** 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 (2.0 mg /kg body wt primaquine), Group IV (5.0 mg /kg body wt primaquine) and Group V (200 mg /kg body wt curcumin) were tested individually for their antimalarial activity. In Group VI animals, antimalarial activity of combination of orally administered curcumin and primaquine was tested (2.0 mg /kg body wt primaquine +200 mg /kg body wt curcumin). Animals were kept under observation to record the mortality and general behavior.

Results

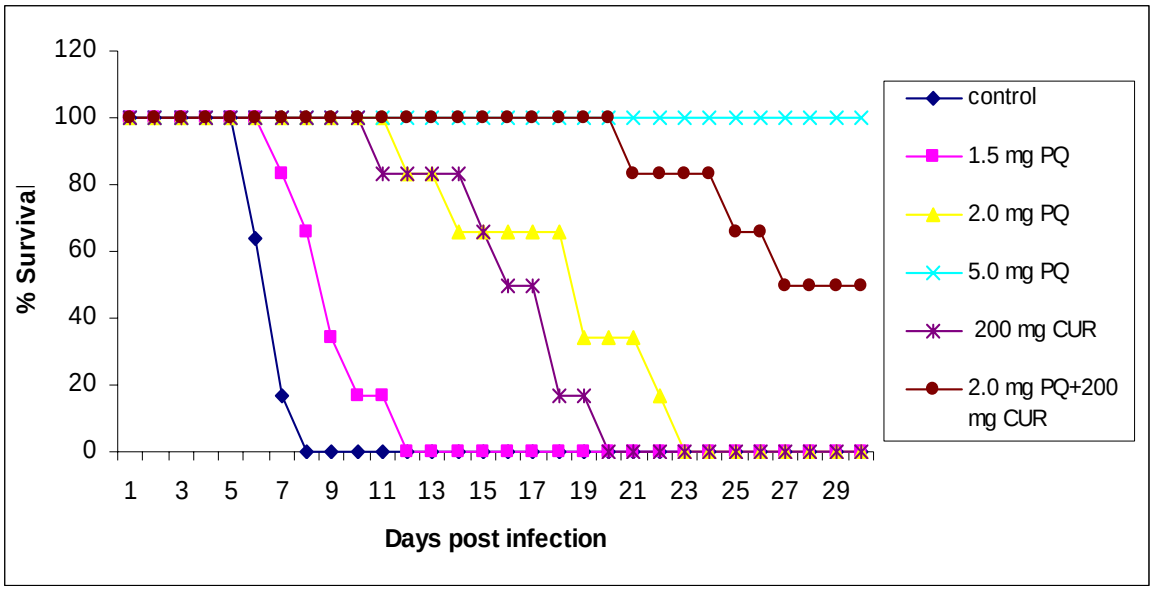
While the *P. berghei*-infected control group animals died between 5 to 8 days. Monotherapy of primaquine at 1.5 and 2.0 mg/kg/day resulted in the death of animals between 7 to 11 days and 12 to 22 days, respectively. For 5 mg/kg/day primaquine treatment animals survived for > 30 days. Treatment of the infected mice with 200 mg/kg/day of curcumin resulted in the survival of mice between 11 to 19 days.

We next tested whether primaquine and curcumin combination therapy could improve survival of infected mice at a low dose of both drugs. Treatment of the infected mice with a combination of primaquine (2.0 mg/kg/day) and curcumin (200 mg/kg/day) resulted in survival of 50 % animals at the end of 30 day study. The results are given in Fig.1.

Discussion

Even though combination therapy is the most preferred therapy to overcome the drawbacks associated with monotherapy, major challenges exist in the deployment and use of this antimalarial drug combination therapies and others include: 1) the cost of combination therapy 2) the choice of drug combinations best suited for the different epidemiological situations 3) the timing of the introduction of combination therapy 4) the operational obstacles to implementation, especially compliance.

Fig. 1. Effect of curcumin and primaquine therapy on mortality of *P. berghei*- infected mice.



PQ : Primaquine, CUR: Curcumin

As a response to increasing levels of antimalarial resistance, the World Health Organization (WHO) recommends that all countries experiencing resistance to conventional monotherapies, should use combination therapies (8).

This study demonstrated that both primaquine at 1.5 and 2.0 mg/kg/day alone and curcumin alone at 200 mg/kg/day mouse increases the survival period of the infected mice compared to control group but all mice died due to malaria infection after the 30 day time period. The only treatment regimen where mice survived beyond 30 days was 5 mg/kg/day primaquine. However, the combination of 2 mg/kg/day primaquine with 200 mg/kg/day curcumin resulted in an overall survival rate of 50% compared to 0% in mice treated with each drug alone by day 30 post-infection. Being a preliminary evaluation of antimalarial activity of this combination of drugs, parasitemia suppressive test was not evaluated.These results indicate that though curcumin and primaquine monotherapy at the indicated doses exhibit antimalarial activity and prolong the survival of *P. berghei*-infected mice, it does not confer complete protection. Combination of primaquine with curcumin results in improved survival rate and appears to be an alternative when compared to monotherapy. Even though the antimalarial activity of curcumin was established earlier this is the first study to demonstrate *in vivo* antimalarial activity of the combination of curcumin and primaquine (6, 7). The exact mechanism of curcumin as an antimalarial drug is not clear at this point and it may act on the hypnozoite stages of *P. berghei*. A possible mechanism of curcumin-induced parasite suppression may be due to its immunomodulatory effect. From literature, curcumin is known for enhancing CD4+ and B cell proliferation that plays an essential role in suppressing parasite by increasing Th1 and Th2 proliferation (9-11). However the protective immunity itself may not be sufficient to clear the parasitemia. In the fixed dose combination of primaquine with curcumin, it is possible that primaquine will reduce parasite load, whilst the curcumin

will clear the remaining parasites by modulating the immune system thereby preventing recrudescence. We propose the hypothesis could be tested further with immunological studies to acquire more insight. From earlier studies it has been shown that curcumin was able to reduce hemolysis caused by the primaquine. Hence this combination, not only enhance the antimalarial activity of each drug but also help to reduce the toxicity caused due to primaquine treatment (12).

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