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Curcumin for malaria therapy

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Abstract

Malaria remains a major global health concern. New, inexpensive, and effective antimalarial agents are urgently needed. Here we show that curcumin, a polyphenolic organic molecule derived from turmeric, inhibits chloroquine-resistant *Plasmodium falciparum* growth in culture in a dose dependent manner with an IC_{50} of ~5 μM. Additionally, oral administration of curcumin to mice infected with malaria parasite (*Plasmodium berghei*) reduces blood parasitemia by 80–90% and enhances their survival significantly. Thus, curcumin may represent a novel treatment for malarial infection.

Keywords: Curcumin; Malaria; Plasmodium; Artemisinin

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. Likewise, the use of antimalarials such as artemisinin, though effective in global malaria control programs, is hampered by high cost and limited supply [1]. Therefore, identification of an antimalarial drug that is easy to produce/isolate, is inexpensive, and demonstrates little toxicity across a diverse population represents the ideal agent needed for global malaria control programs and eradication of this deadly disease. Curcumin isolated from the roots of the *Curcuma longa* plant has been shown to regulate a number of biological responses [2]. In addition to its anti-tumorigenic, anti-oxidant, and anti-inflammatory effects, curcumin has been shown to possess anti-microbial activity [2,3]. In addition to inhibiting the growth of a variety of pathogens, curcumin has been shown to hinder *Leishmania* [4] and *Trypanosoma* [5] viability. With demonstrated antiprotozoal activity, we hypothesized that curcumin would similarly affect malarial *Plasmodium* parasitic infection. This study was undertaken to determine the antimalarial effects of curcumin using well-standardized and characterized in vitro and in vivo systems.

Materials and methods

Reagents. Curcumin (Sigma Chemical, St. Louis, MO) was dissolved in 100% dimethyl sulfoxide (DMSO; Sigma Chemical) and stored in aliquots at −20 °C.

Parasite maintenance. Plasmodium falciparum culture was maintained by the candle jar method [6] using human O positive serum and red blood cells. *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 in PBS and 0.1 ml was injected intraperitoneally into fresh mice for parasite propagation. The treatment of mice and all experimental protocols were according to the Institutional Animal Care and Use Committee guidelines.

In vitro and in vivo antimalarial activity of curcumin. In vitro antimalarial activity was assayed by measuring [^{3}H]hypoxanthine incorporation into a chloroquine resistant *P. falciparum* strain isolated locally [7]. In vivo antimalarial activity was examined in groups of 10–15 male Swiss mice (40–50 g) intraperitoneally infected on day 0 with

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P. berghei such that all the control mice died between day 8 and day 10 post-infection. Curcumin was dissolved in DMSO (100 mg/kg body weight) and was presented at the tip of an Eppendorf pipette into the oral cavity of non-anesthetized mice as five consecutive daily doses. Curcumin-treated uninfected mice and vehicle-treated *P. berghei*-infected mice served as controls. Survival of mice was monitored for a period of 3 weeks.

Statistical analysis. An unpaired, two-tailed Student's *t* test was used to analyze in vitro data and the log rank test for analyses of survival curves. All calculations were performed on the Prism 3.0 statistical program (Graphpad Software, San Diego, CA). A value of $P < 0.05$ was considered significant.

Results

Using [^{3}H]hypoxanthine uptake to assess viability, we determined the effects of curcumin on a chloroquine-resistant strain of *P. falciparum* carrying the PfCRT-K76T mutation, a molecular marker for chloroquine-resistance [7]. Curcumin inhibited the growth of *P. falciparum* in a dose-dependent fashion, with an IC_{50} of ~5 μM. Increasing doses of curcumin resulted in decreased viability of *P. falciparum*, with a dose of 50 μM leading to negligible proliferation (Fig. 1). When tested in a well-characterized in vivo *P. berghei*-rodent infection model, curcumin had significant beneficial effects. Oral feeding of curcumin to *P. berghei*-infected mice decreased blood parasitemia by 80–90% (Fig. 2A) and enhanced their survival significantly compared to vehicle-fed controls ($P < 0.001$) (Fig. 2B). Curcumin treatment resulted in an overall survival rate of 29% compared to 0% in vehicle-fed animals by day 21 post-infection. Curcumin was administered 48 h after infection (3–5% parasitemia), once daily for 5 days at a dose of 100 mg/kg body weight. This dose of curcumin has not been demonstrated to result in toxic side effects in humans when compared on a weight to weight basis [8].

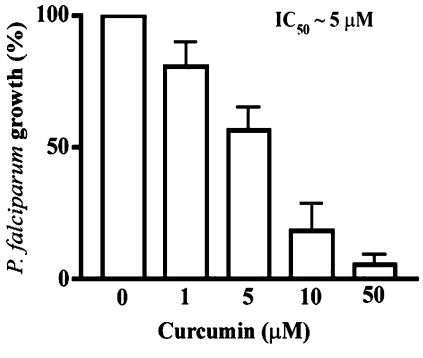

Fig. 1. In vitro antimalarial activity of curcumin. Curcumin inhibits chloroquine-resistant *P. falciparum* growth in culture. Percent [^{3}H]hypoxanthine incorporation into parasite in the absence of curcumin was taken as 100% growth. Assays were done in triplicate and averages from four independent experiments are shown. Error bars indicate standard deviation.

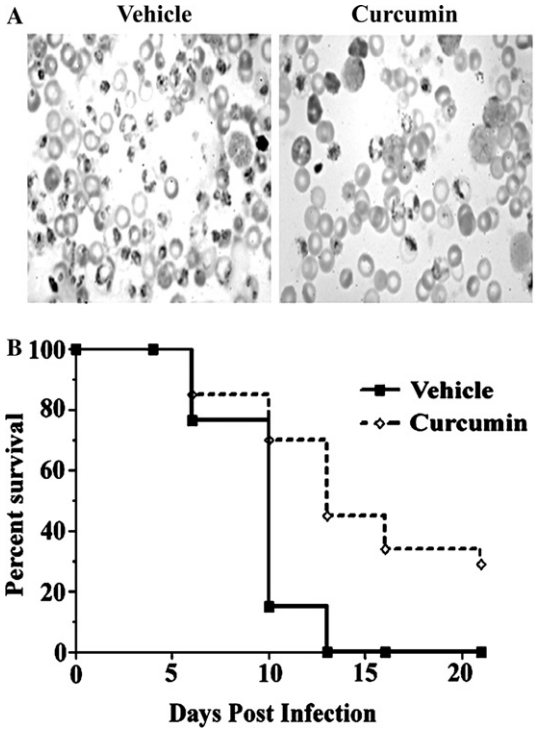

Fig. 2. In vivo antimalarial activity of curcumin. (A) Oral administration of curcumin decreases parasitemia by >80% in *P. berghei*-infected mice. The figure shows Giemsa-stained smears of blood collected from a *P. berghei*-infected mouse fed with vehicle or curcumin between days 9 and 10 post-infection when majority of the vehicle-fed mice began to die. (B) Oral administration of curcumin increases the survival of mice infected intraperitoneally with *P. berghei*. Two days after *P. berghei* infection, mice were orally administered either curcumin (100 mg/kg body weight) or vehicle as control once daily for a period of 5 days. Survival curves were generated from three independent experiments with a total of 10 mice per group.

Discussion

Curcumin, a component of the widely used Ayurvedic compound turmeric, is a potent biological compound. Numerous studies have characterized its effects on a wide variety of cellular processes and diseases. Few studies, however, have extended in vitro antimicrobial observations to in vivo models. Only one study to our knowledge has previously examined the effects of curcumin on *P. falciparum* [3]. In contrast to our study, Rasmussen et al. examined only drug sensitive *P. falciparum* and did not test the use of curcumin in an in vivo infection model. The IC_{50} of curcumin on *P. falciparum* in that study, interestingly, was similar to what we observed using a drug-resistant strain of *P. falciparum*. Further, we demonstrate that curcumin administered orally for 5 consecutive days, 48 h after *P. berghei* infection, reduces the parasite burden by more than 80% and completely protects up to 29% of infected mice. It is pertinent to note that potent antimalarials such as Artesunate and Artemether prolong the survival of *P. berghei*-infected mice only up to 11 and 22.3 days,

respectively, when administered orally for three consecutive days 24 h after infection [9]. Since a phase 1 human trial using up to 8000 mg of curcumin per day for 3 months found no toxicity from curcumin [8], it will be interesting to examine whether prolonged treatment of curcumin can completely eliminate the parasite and prevent recrudescence.

The exact mechanism(s) of curcumin's antimalarial action is not clear. The inhibition of parasite growth in culture suggests a direct mechanism of action involving parasite biochemical processes. One of the possible targets for curcumin action could be PfATP6, the parasite orthologue of mammalian sarcoplasmic–endoplasmic reticulum Ca^{2+}-ATPase (SERCA). It is known that curcumin inhibits mammalian SERCA with an IC_{50} of 7–15 μM [10] which correlates well with the IC_{50} values (~5 μM) observed with *P. falciparum* in this study. Artemisinin has recently been shown to inhibit PfATP6, an orthologue of SERCA in *P. falciparum* (K_i ~150 nM) [11]. It is therefore possible that curcumin and artemisinin act through similar mechanisms.

Curcumin appears to be an ideal antimalarial molecule especially for use in combination with antimalarials such as artemisinin not only to limit the use of the latter but to overcome the problems of high cost, recrudescence, and drug resistance. In view of its abundance, non-toxic nature, and demonstrated therapeutic effects in a variety of human diseases, it will be useful to further investigate the potential of curcumin in developing low-cost antimalarial therapies.

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