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Plasmodium falciparum: in vitro growth inhibition by febrile temperatures

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Abstract Febrile episodes are the hallmark of malarial infection. We determined the inhibitory effect of febrile temperatures on the in vitro growth of *Plasmodium falciparum*. Parasites were cultured at various temperatures between 37 °C and 40 °C for 4 days. A logistic decrease in parasitaemia as a function of temperature was observed for continuous cultures. Incubation of synchronized cultures for different lengths of time during the parasite cycle showed a strong increase of growth inhibition with the maturing of parasites. Febrile temperatures inhibit parasite growth and long, high fevers during malaria may be beneficial for parasite clearance.

Introduction

Plasmodium falciparum malaria is a life-threatening infectious disease. World-wide, 1.5 to 2.7 million people die from severe malaria each year, the vast majority being children under 5 years of age in sub-Saharan Africa (World Health Organisation 1995). The most characteristic clinical feature of malaria is the febrile episode, probably caused by rupturing schizonts. An in vitro study has shown that febrile temperatures of 40 °C inhibited the development of *P. falciparum*, with maximal effect in the second half of its 48-h replicative cycle (Kwiatkowski 1989). However, the role of fever in protection against malaria remains to be shown decisively (Kwiatkowski 1995).

This study was designed to elucidate the association between febrile temperatures and parasite growth in vitro. We investigated the growth of *P. falciparum* in continuous culture at various temperatures. Furthermore, we tried to determine the parasite stages most sensitive to temperature.

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Materials and methods

Parasite isolates

A laboratory-adapted isolate of *P. falciparum,* BINH, was used in this study (Binh et al 1997). The parasites were cultured according to a modification of the method of Trager and Jensen (1976). The culture medium consisted of RPMI 1640 medium with glutamine (Seromed, Berlin, Germany), 25 mM HEPES (Seromed), 28 mM sodium bicarbonate (Merck, Darmstadt, Germany), 40 μM hypoxanthine (Serva, Heidelberg, Germany), 10 mM glucose (Merck) and 40 mg/l of gentamicin (Gibco-BRL, Paisley, UK). Before use, the medium was completed by the addition of 1.5 g/l of Albumax, a lipid-rich bovine albumin (Gibco-BRL).

Wild parasite isolates were obtained from cryopreserved blood of Gabonese patients with malaria.

Parasite cultures

The parasites were synchronized by two cycles of 5% sorbitol lysis according to the technique of Lambros and Vanderberg (1979). After synchronization, thin smears were made and stained with Giemsa, to ensure that the parasites were well synchronized and in the young ring-form. *P. falciparum* cultures with an initial parasitaemia of 1.5% and haematocrit of 2% were put in culture immediately after synchronization with a final volume of 6 ml in a 50 ml flask. The cultures were aerated in a gaseous mixture of 5% O_2, 5% CO_2 and 90% N_2 and incubated at continuous temperatures of either 37 °C, 38 °C, 38.5 °C, 39 °C, 40 °C or 41 °C for 4 days. To determine the sensitivity of elevated temperatures as a function of parasite age, the parasites were exposed to either 39 °C or 40 °C for 6 or 12 h at different starting times and subsequently cultured at 37 °C for a total of 4 days, counting from the start of the culture. All experiments were performed 4 times, each with a medium change every 2 days.

Growth monitoring

Aliquots (200 µl) from every culture flask were obtained daily, centrifuged and the pellets were conserved in 0.25% glutaraldehyde. Parasitaemia was determined by fluorescence-activated flow cytometry (FACS). For FACS analysis, pellets from cultures were stained with 0.25% acridine-orange for 5 min. All incubations and washing steps were performed in phosphate-buffered saline. The fluorescence values of 10,000 cells per sample were evaluated using a FACScan flow cytometer (Becton Dickinson) with a theoretical error of 0.01% in parasitaemia. Giemsa-stained thin films were made for morphological monitoring.

Statistical analysis

The parasitaemias at elevated temperatures were divided by the corresponding parasitaemias at 37 °C taking into account the proper culture flask and day. For the analysis of variance these ratios were log-transformed in order to stabilize variances. The expected means and their 95% confidence limits of the logarithms were subsequently back-transformed. In order to improve the estimate of the standard deviation of the residuals, the data for all days were included in the analysis.

Results

The growth of *P. falciparum* was reduced at constant elevated temperatures.

Microscopic observation showed that the development of the parasites stopped at late trophozoite and schizont stages within the first growth cycle at temperatures above 40 °C. The schizonts appeared pycnotic and hyposegmented and the parasites failed to develop into a new cycle. At 39 °C, the thin smears of the cultures showed both well-grown and pycnotic schizonts. Figure 1 shows the effect of different constant temperatures. Significantly reduced parasitaemia was observed at 39 °C when compared to the parasitaemia at 37 °C. At 38.5 °C, parasite growth was also significantly inhibited ($P = 0.05$). At 38 °C, no significant difference to 37 °C could be observed in the parasite growth and morphological appearance, even though a slight reduction was found in the FACS analysis. A logistic model was fitted to the observed ratios of parasitaemia as a function of the logarithm of the difference between the applied temperature and 37 °C. In this model, a 50% reduction in parasitaemia is predicted at 38.8 °C. The reduction is 92.4% for 40 °C and 98% at 41 °C. This model explains 82% of the variance.

Immediately after thawing, three wild isolates from Gabon were cultured at 37 °C and 39 °C for 4 days and compared to the unsynchronized parasite laboratory-isolate BINH. The effects of hyperthermic conditions on parasite growth on wild isolates were similar to those obtained with the laboratory-adapted strain (data not shown).

The parasites were most heat susceptible during late stages of their development. A 12-h treatment at 39 °C significantly inhibited growth only for parasites older than 12 h (Fig. 2). At 40 °C, parasites were inhibited at all stages. A 6-h treatment at 39 °C did not affect the parasites at any stage of the cycle, whereas this treatment led to growth inhibition at a temperature of 40 °C, for parasites aged 30 h or more (Fig. 3).

Independent of incubation time, there was an exponential increase in growth inhibition at 40 °C as a function of parasite age, starting at the beginning of the parasite cycle. Analysis was also done by calculating parasite multiplication rates between start of incubation and 48 h. A multiplication rate of 5.0 was found at 37 °C and this was significantly reduced at higher temperatures. This method confirmed the results obtained by analysis of the growth rates mentioned above.

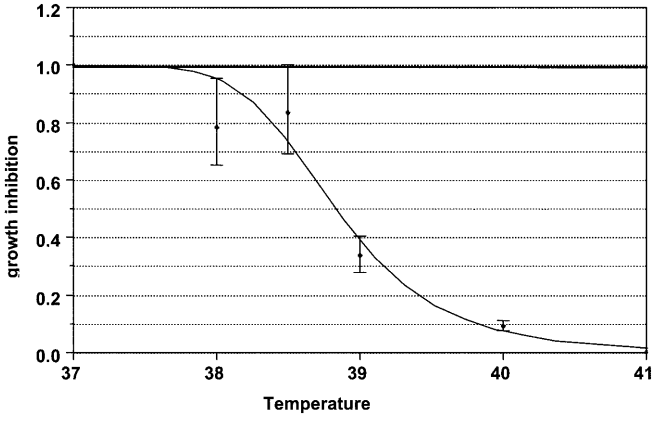

Fig. 1 The effect of constant febrile temperatures on the growth of *Plasmodium falciparum*. A logistic model explains the growth inhibitory effect

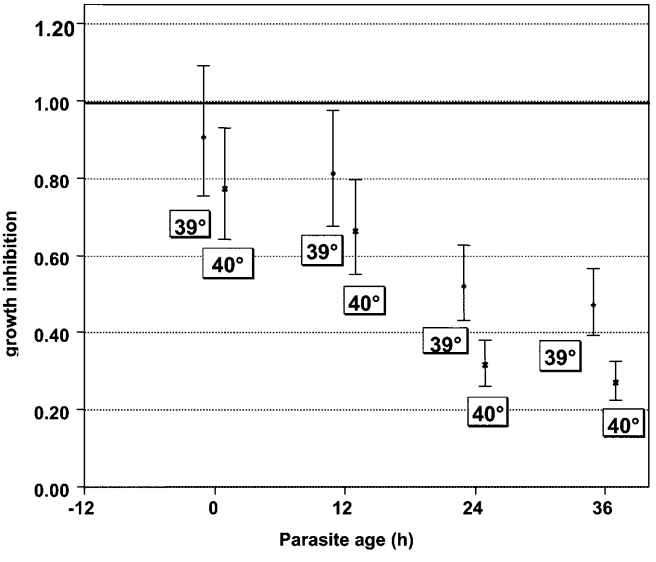

Fig. 2 Growth inhibition of *P. falciparum* exposed to 12 h of febrile temperatures beginning at various parasite ages. The greatest effect is seen in older parasites

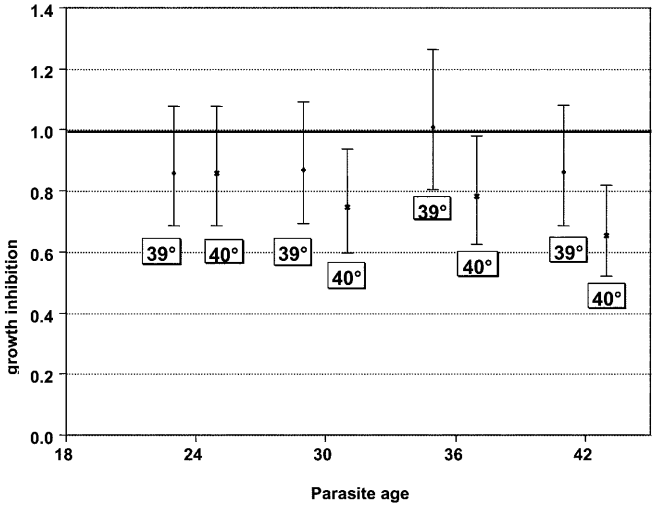

Fig. 3 Growth inhibition of *P. falciparum* exposed to 6 h of febrile temperatures beginning at various parasite ages. An effect is only seen in older parasites and at 40 °C

Discussion

Fever is a fundamental host defence mechanism that does not require prior exposure to the parasite or immunological memory. Animal studies have shown both the beneficial effect of fever and the negative effect of antipyretics in the course of infectious diseases (reviewed in Kluger et al. 1997). Clinical studies in humans suggest a beneficial effect of fever on sepsis (Mackowiak et al. 1980) and bacterial peritonitis (Weinstein et al. 1978) and a negative effect of treatment with antipyretics in rhinovirus infections (Graham et al. 1990). Malaria, which induces high and long-lasting fevers, would seem to be an ideal candidate for evaluating the effect of fever on the course of infection. However, the relationship between fever and *P. falciparum* parasitaemia is complex. The growth of *P. falciparum* parasites in the peripheral blood is not synchronized, making it difficult to model the relationship between parasitaemia and body temperature, in contrast to other species such as *P. vivax* (Karunaweera et al. 1992). The modelling of the course of parasitaemia in individuals must take into account both the effect of the parasite on body temperature as well as the effect of fever on the parasite. Surprisingly little is known about the effect of elevated temperatures on the growth of *P. falciparum* in vitro and, up to now, only one study has focused on this question (Kwiatkowski 1989). However, since only one single temperature (40 °C) was evaluated, this study cannot calculate the effect of temperature on parasites at other temperatures. Furthermore, the cultures used in this study might have been contaminated with mycoplasmas (Rowe et al. 1998).

A comparison between this study and the present one shows a similar estimation of the multiplication rate, thus allowing direct comparison of the data. Both show that parasite growth is effectively inhibited by temperatures which occur during febrile episodes in patients. In addition, our study shows that, at least at high temperatures, plasmodia are susceptible even in the early trophozoite stage. This is in contrast to the results of Kwiatkowksi (1989), who found no effect of elevated temperature on these young parasites. This finding has implications for models of parasite population dynamics which try to estimate the effect of fever on the course of a *P. falciparum* infection (Gravenor and Kwiatkowski 1998). Our data suggest that present models may underestimate the antiparasitic effect of febrile temperatures during an acute episode of *P. falciparum* malaria.

Temperatures around 40 °C for several hours are not uncommon in clinical practice. Long-lasting high body temperatures should lead to a faster reduction of parasites in patients with a high number of late-stage parasites. Our results confirm the theory of a protective effect of fever and shed some further doubts on antipyretic therapy during malaria.

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