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Effect of heat-shock on *Plasmodium falciparum* viability, growth and expression of the heat-shock protein 'PFHSP70-I' gene

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Cultures of the human malaria parasite *Plasmodium falciparum* were subjected to heat-shock for varying times and temperatures and then tested for their viability, growth and expression of heat-shock protein. Results show that the majority of parasites remained viable after heat-shock but their growth was affected. However, the expression of the heat-shock protein 'PFHSP70-I' gene was enhanced after heat-shock. We conclude that malarial parasites are able to survive in vivo during fever probably due to the overexpression of the heat-shock protein gene.

Heat-shock protein; Gene expression; Human malaria parasite; Fever

1. INTRODUCTION

Plasmodium falciparum undergoes a heat-stress during malaria fever. Several stress proteins of this parasite have been reported [1]. Among them the heat-shock protein of 75 kDa named 'PFHSP70-I' has been studied in greater detail [2–5]. This protein has also been proposed for a malaria vaccine because: (a) it is expressed in abundant quantities during the liver stage and all of the erythrocytic stages; (b) it is probably expressed at the merozoite's surface; and (c) monkeys were protected against malaria after immunization with a 75 kDa protein band of *P. falciparum* [2–8]. The functional role of this protein is not yet clear [1,9,10]. Therefore, the present study describes the effect of various body temperatures (close to malaria fever) on the *P. falciparum* viability, growth and expression of the PFHSP70-I gene.

2. MATERIALS AND METHODS

2.1. *Effect of various temperatures on in vitro growth of* P. falciparum

The *P. falciparum* parasites were grown in the laboratory using the candle jar method [11] and synchronized to rings by the method of Biswas et al. [12]. The synchronized cultures were pelleted down by centrifugation and then adjusted to 10% hematocrit. A 400 μl aliquot of this suspension was transferred to the 1.5 ml microfuge tubes. These tubes were exposed to 35, 37, 39 and 41°C temperatures individually for 2, 4, 6, 8, 10, 20, 30 and 60 min. After heat-shock the parasites in a 100 μl aliquot from each tube, in duplicate, were transferred to 96-well microtiter culture plates and then grown further for 48 h at 37°C. Slides for light microscopy were made just after heat-shock (day 0) and after 48 h of incubation at 37°C (day 2). Parasites were counted in a field of 100 cells. A total of 5,000 cells were counted.

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2.2. *Effect of heat-shock on PFHSP70-I gene expression*

2.2.1. Isolation of RNA

Total RNA from the parasite was prepared as described earlier [13]. Briefly, the parasites grown at 37°C in culture, with and without heat-shock, were collected after saponin lysis. The parasite pellet was dissolved in a buffer solution containing 4 M guanidinium isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarcosyl, 0.1 M 2-mercaptoethanol and 2 M sodium acetate. The RNA was extracted by vortexing in a mixture of phenol/chloroform/isoamyl alcohol. The aqueous phase containing total RNA was separated by centrifugation at 4°C for 20 min at 10,000 × *g* and RNA precipitated with isopropanol at −20°C. It was centrifuged and the RNA pellet, after washing and drying, was redissolved in diethyl pyrocarbonate treated 0.5% SDS.

2.2.2. RNA dot-blot hybridization

The RNA in 0.5% SDS was diluted 3-fold with formamide and formaldehyde in 20 × SSC (20:7:2 v/v). The mixture was heated at 68°C for 15 min and then quenched on ice. To this was added 2 vols. of 20 × SSC and it was then loaded to the nitrocellulose filter. This filter was hybridized with the previously isolated cDNA clone (HS4) which encodes for the C-terminal half of the *P. falciparum* HSP70-I [2]. The hybridization and washing conditions were the same as described therein [2].

3. RESULTS AND DISCUSSION

3.1. *Effects of heat-shock on parasite growth*

The cardinal symptom of human malaria is fever which is associated with the rupture of intra-erythrocytic schizonts. Here, we have attempted to study the effects of varying temperatures (35–41°C), similar to malaria fever, on the viability and in vitro growth of the erythrocytic stages of *P. falciparum*. The synchronized cultures were exposed to these temperatures for certain periods of time (2–60 min) and then returned to 37°C for a further 48 h incubation. The latter (further allowance of parasite growth at 37°C for 48 h) was to mimic

Table I

Effect of heat-shock on *P. falciparum* viability

Time of exposure (min)	Number of parasites per 5,000 RBCs											
	35°C			37°C			39°C			41°C		
	R	T	S	R	T	S	R	T	S	R	T	S
0	88	10	5	88	10	5	88	10	5	88	10	5
2	80	15	5	88	10	5	83	18	0	72	3	0
4	78	22	3	87	10	5	82	15	0	65	3	0
6	80	18	5	78	18	10	83	13	0	55	3	0
8	80	15	7	85	17	5	83	10	0	58	0	0
10	85	15	0	83	22	3	85	12	0	62	0	0
20	85	20	0	90	10	0	85	5	0	60	0	0
30	75	5	0	95	10	2	75	5	0	55	0	0
60	65	0	0	85	15	5	65	0	0	45	0	0

The parasites were exposed to various temperatures for varying times. The light microscopy slides were made and the different blood stages of the live parasites were counted. The dead parasites were not included since they had disintegrated and therefore the stages were not distinguishable. R = ring; T = trophozoite; S = schizont.

the in vivo conditions of recurrence of intermittent malaria fever episodes. The inclusion of 35°C in our experiments was based on the fact that during post-malaria fever the abundant perspiration brings down the body temperature to a suboptimal level. The results of these experiments are shown in Table I, which indicate that the majority of the parasites remained viable after heat-shock. We observed that schizonts were most vulnerable to heat-shock since they were not seen in the samples exposed to 39°C or 41°C. Schizonts were also missing from the cultures which were exposed to 35°C for 10 min or longer. Although trophozoites were not affected as adversely as schizonts, they were also not seen in the cultures exposed to 41°C for 8 min or longer. Rings, however, seemed to be least affected. A similar situation in vivo could lead to the synchronization of the parasites and therefore the giving rise to the characteristic intermittent malaria fever.

Although the majority of the parasites remained viable after the heat-shock, their growth was certainly affected (Fig. 1A,B). Fig. 1A shows that parasites grew faster in control culture (without a heat-shock) at 37°C compared to those which were exposed to 35°C, 39°C or 41°C for certain periods of time before returning them to 37°C for 48 h. The maximum adverse effects were observed in the cultures which were exposed to 41°C. Fig. 1B shows that the maximum number of parasites were found dead after a heat-shock at this temperature; the death rate was increased if they were exposed for 30 min or longer. The parasite death rate also remained high after their (heat-shocked at 41°C) further growth at 37°C for 48 h. After such high fever (41°C) the parasite would like to escape such an endangered host. For this reason, it could be transformed into the transmittable stage of gametocytes rather than multiplying and growing further into other blood stages. This type of transformation could be related to the expression of certain stress proteins as noticed in the case of *Leishmania* where the heat-shock protein is implicated in the transformation of promastigotes to the infective stage of amastigotes [14]. Since our cultures do not produce gametocytes, it was thus difficult to test this hypothesis. However, the predominance of rings and trophozoites in a 41°C heat-shocked culture grown at 37°C for 48 h, is also an indication in this direction. Earlier we have shown that the expression PFHSP70-I gene was maximum at ring stage [2].

3.2. *Effect of heat-shock on PFHSP70-I gene expression*

Total RNA, isolated from control and heat-shocked (39°C for 30 min) parasites were hybridized with the cDNA probe of PFHSP70-I [2]. The results shown in Fig. 2 indicate that the transcriptional signals for PFHSP70-I were higher from the 39°C exposed parasites compared to those of the 37°C controls. The enhanced expression of the PFHSP70-I gene after heat-shock indicates that this protein belongs to a class of heat-shock proteins (HSPs). Nevertheless, the PFHSP70-I is also expressed constitutively at 37°C [2,7,8]. The present study, therefore, suggests that the PFHSP70-I gene is heat inducible.

The expression of the PFHSP70-I gene described here should be quite specific since under these stringent hybridization conditions the other members of the PFHSP70 gene family do not show cross-reactivity [15] although they share a large amount of sequence homology [1]. Also, these PFHSP70 family proteins are antigenically distinct from each other [15]. However, it will

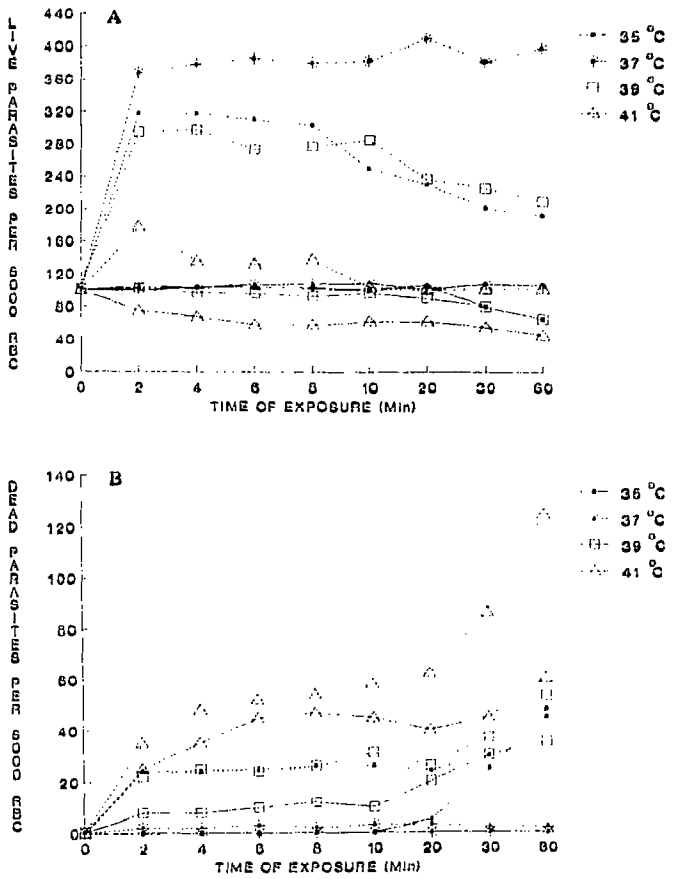

Fig. 1. Effect of various temperatures on the *P. falciparum* parasite growth. The parasites were exposed to various temperatures for varying times and slides were made to count the number of live (A) and dead (B) parasites. This is shown by the solid lines in the figure and indicated as day zero in the text. These parasites, after heat-shock, were then allowed to grow further for 48 h at 37°C. The live (A) and dead (B) parasites were again counted and are shown as broken lines in the figure and indicated as day 2 in the text.

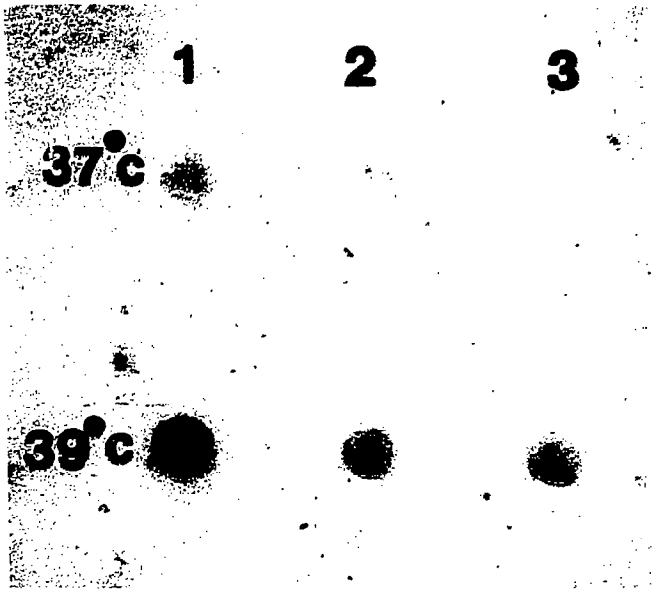

Fig. 2. Effect of heat-shock on the expression of the PFHSP70-I gene. For RNA dot-blot analysis, total RNA was isolated from heat-shocked (39°C) and non-heat-shocked (37°C) *P. falciparum* parasites and loaded to the nitrocellulose filter (lane 1: 6 μg; lane 2: 3 μg; and lane 3: 1.5 μg). The blot was hybridized with the cDNA (HS4, coding for C-terminal half of the PFHSP70-I) probe under the described conditions [2].

be interesting to know if *P. falciparum* also synthesizes the various isoforms of HSP70 as recently described for *Trypanosoma cruzi* [16]. In this latter case, the heat-shock induced the expression of several HSP70 isoforms possibly via preferential translation of pre-existing mRNA. However, this phenomenon of isoform formation is probably linked to the organization and thus the expression of the HSP70 gene family; the HSP70 genes in *Trypanosoma* are clustered and transcribed as polycistronic mRNA. It is, therefore, less likely that a similar situation occurs in malaria because the HSP70 genes of *P. falciparum* are located on different chromosomes, therefore, their inheritance and expression is independent of each other [15–18].

The enhanced transcription of PFHSP70-I could be associated with the fever in vivo. During malaria fever the body temperature increases for sometime (heat-shock for the parasite) which could trigger the high level expression of the PFHSP70-I gene. The enhanced expression of PFHSP70-I could be essential to protect the parasite from killing at the higher temperatures encountered during malaria fever, as well as getting it transformed into the transmittable stage of gametocytes to escape the host. The mechanisms of enhanced transcription could be similar to the other models where heat-shock factors bind more tightly to the heat-shock elements present in upstream regions of the gene because of some structural changes occurring in the factor at higher temperatures [19].

We conclude that the majority of the parasites remain viable at different body temperatures, which are encountered by the patients during falciparum malaria fever, but their growth is retarded. The higher temperatures could also be responsible for the retarded growth. The higher temperatures could also be responsible for the synchronization of the parasites thus resulting in the characteristic recurrence of intermittent malaria fever. The exposure of the parasite to higher temperatures during fever leads to the enhanced synthesis of the PFHSP70-I transcript. The enhanced expression of PFHSP70-I may protect the parasite from killing by heat and possibly transforms it to a more heat-resistant and transmittable stage.

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