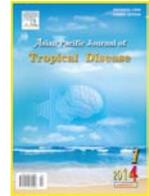




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Expression of heat shock protein 90, 70, 60 and 25 in the placenta of *Plasmodium berghei* infected BALB/c mice

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PEER REVIEW

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Comments

This is an excellent study where in authors have demonstrated the expression of hsps in placenta of malaria infected mice. Results showed down regulation of hsps 70, 60 and 25 with progression of infection, which suggested the importance of hsps in combating the placental pathological alterations due to malarial infection. It is very interesting.

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ABSTRACT

Objective: To assess the role of heat shock proteins (hsps) in the placental pathology of *Plasmodium berghei* infected mice.

Methods: Female BALB/c mice were infected with *Plasmodium berghei* infected RBC intraperitoneally on 10th gestational day. The expression of hsps in the placenta were monitored by reverse transcriptase real time PCR.

Results: There was increased expression of hsps 90, 70, 60 and 25 genes on day 2, 4 and 6 post infection in the infected placenta. However, the expression of hsps 70, 60 and 25 genes decreased with the progression of malaria infection.

Conclusions: Decreased expression of hsps 70, 60 and 25 genes in the malaria infected placenta might be one of the many other factors responsible for apoptosis in the placenta and may be responsible for associated altered placental pathology and fetal abnormalities.

KEYWORDS

Plasmodium berghei, Pregnancy, Placenta, Heat shock proteins

1. Introduction

Malaria with over 3 million deaths per year remains the foremost killer among all diseases and causes 400 000 cases of severe maternal anaemia and from 75 000–200 000 infant deaths annually in sub-Saharan Africa[1]. Malaria is more severe in pregnant women than nonpregnant women leading to altered placental pathology and fetal abnormalities[2]. The role of heat shock proteins (hsps) in infection and immunity is receiving much attention.

Increased expression and accumulation of the hsps during infections facilitate the ability of cells to both repair and synthesize new proteins[3,4]. In our earlier studies, we have reported that *Plasmodium berghei* (*P. berghei*) infection in pregnant mice leads to enhancing oxidative stress that induces mitochondrial mediated pathway of apoptosis in the placenta[5,6]. Thus, in the present study an attempt was made to correlate the expression of hsps with placental pathology in *P. berghei* infected pregnant BALB/c mice.

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2. Materials and methods

2.1 Animals

Female BALB/c mice ($n=6$), 6–8 weeks old were infected on gestational day 10 with 1×10^6 *P. berghei* (NK 65) infected RBC intraperitoneally. However, mice from control group ($n=6$), were intraperitoneally inoculated with normal saline on gestational day 10[5,6]. Mice were sacrificed by cervical dislocation on day 2, 4 and 6 post infection and placentae removed. Care, use and disposal of animals were done in accordance with the guidelines of Institutional Animal Ethical Committee (44/99/ CPCSEA).

2.2. Reverse transcriptase real time PCR

RNA from placenta was isolated using TRI reagent (Sigma Aldrich) following manufacturer's protocol. Purity and integrity of RNA was monitored in 1.5% agarose ethidium bromide gel and quantitated at A260 using the Nano-Drop 1000 spectrophotometer (Thermo Scientific). From RNA complementary DNA was synthesized using commercially available kit (Fermentas Life Sciences). From complementary DNA, real time PCR was performed using DyNAmo ColorFlash SYBR Green qPCR kit (Thermo Scientific) and following PCR programme: 95 °C for 7 min for initial denaturation; then 40 cycles of denaturation at 94 °C for 10 seconds; annealing at 54.2 °C for 30 seconds; elongation at 68 °C for 30 seconds and final elongation at 68 °C for 7 min. Following sets of primers were used in the study: hsp90–Forward primer 5'AGC TTT CAG AGC TGT TGC GGT 3', Reverse primer: 5' TTG CTG TCC ACC CAT ATG TGC 3'; hsp70–Forward primer: 5'GCC AAG GCC AAC AAG ATC A 3', Reverse primer: 5'AGA TGA CCT CCT GGC ACT TGT C 3'; hsp60–Forward primer: 5'AAA ATT TGG TGC GGA CGC T3', Reverse primer 5'AAA GCC CTC CTT GGC AAT AGA T 3'; hsp25–Forward primer: 5'GCA GGA CGA ACA TGG CTA CAT 3', Reverse primer: 5'GGC CTC GAA AGT AAC CGG AAT 3'; β -actin Forward primer: 5'ATG GAA TCC TGT GGC ATC CA 3', Reverse primer 5' TCC ACA CAG ACT ACT TGC GCT C 3' (procured from Genex Life Sciences Pvt. Ltd, Bangalore, India). The data was analyzed as described by Livak and Schmittgel[7] and results expressed as mean \pm standard deviation. The inter group variation was assessed by two way analysis of variance (ANOVA) and statistical significance was calculated at $P<0.05$.

3. Results

The expression of hsp90, 70, 60 and 25 genes in the

infected placenta at each point of observation compared with normal placenta. However, expression of hsp90, 60 and 25 decreased significantly ($P<0.05$) in the malaria infected placenta on day 4 and 6 post infection compared with day 2 post infection (Figure 1).

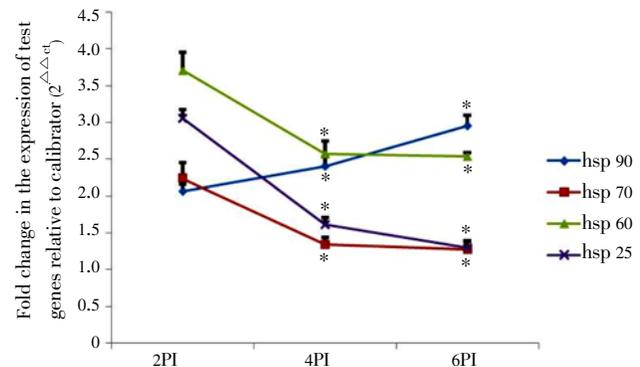


Figure 1. Expression of hsp90, 70, 60 and 25 genes in the *P. berghei* infected placenta on day 2, 4 and 6 PI compared with non-infected placenta. Values are expressed as mean \pm standard deviation. * ($P<0.05$) vs. 2 post infection.

4. Discussion

The materno–fetal interface, placenta is the potential site for the expression of high levels of hsp90 which are important for normal functioning of the placenta[8,9]. In the present study, expression of hsp90 genes in the malaria infected placenta and its contribution to the placental pathology were assessed.

The data of present study demonstrated that malarial infection in pregnant mice enhanced the expression of hsp90, 70, 60 and 25 genes in the infected placenta at each point of observation compared with normal placenta suggesting the protective role of hsp90. This observation is in concordance with earlier studies where over expression of hsp90 have been found to protect against oxidative stress in preeclampsia and TNF–induced lethal inflammatory shock[10,11]. However, the expression of hsp70, 60 and 25 decreased significantly in malaria infected placenta with progression of infection. In our earlier studies, we have found that malarial infection in pregnant mice results into oxidative stress induced mitochondrial pathway of apoptosis and in the present study decreased expression of hsp90 might be one of many other factors responsible for apoptosis and altered placental pathology[5,6]. Thus, it can be concluded that the altered placental pathology during malarial infection is a complex process and may be involving physiological, molecular and immunological mechanisms leading to poor pregnancy outcomes. However, in depth clinical study is needed to understand the role of hsp90 in modulating the placental pathology.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The financial assistant provided by Indian Council of Medical Research (F/826/2011–ECD–II), New Delhi, India to carry out the present work is highly acknowledged.

Comments

Background

Malaria with over 3 million deaths per year remains the foremost killer among all diseases and causes 400 000 cases of severe maternal anaemia and from 75 000–200 000 infant deaths annually in sub-Saharan Africa. Malaria during pregnancy leads to altering placental pathology and fetal abnormalities. In our earlier studies, we have reported that *P. berghei* infection in pregnant mice leads to enhanced oxidative stress that induces mitochondrial mediated pathway of apoptosis in the placenta.

Research frontiers

The role of hsps in infection and immunity is receiving much attention. Therefore, in the present study an attempt was made to correlate the expression of hsps with placental pathology during malarial infection.

Related reports

Hsps 70 and hsp 65 have been found to protect against TNF-induced lethal inflammatory shock and *Toxoplasma gondii* infection in mice. Similarly, in the present study enhanced expression of hsps 90, 70, 60 and 25 genes have been found in the malaria infected placenta compared with normal placenta which suggest the protective role of hsps.

Innovations & breakthroughs

Present study, demonstrated the decreased expression of hsps 70, 60 and 25 on day 4 and 6 compared with day 2 post infection in the malaria infected placenta and might be one of many other factors responsible for apoptosis and altered placental pathology.

Applications

The study has provided better insight into the placental pathology during malarial infection. However, in depth

clinical study is needed to understand the role of hsps in modulating the placental pathology

Peer review

This is an excellent study where in authors have demonstrated the expression of hsps in placenta of malaria infected mice. Results showed down regulation of hsps 70, 60 and 25 with progression of infection, which is very interesting and suggested the importance of hsps in combating the placental pathological alterations due to malarial infection.

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