HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2015.03.007

Effect of pre-existing *Schistosoma haematobium* infection on *Plasmodium berghei* multiplications in imprinting control region mice

Benjamin Amoani[1,2*], Elvis Ofori Ameyaw[1], Du-Bois Asante[1], Francis Ackah Armah[1], James Prah[3], Collins Paa Kwesi Botchey[1], Johnson Nyarko Boampong[1]

[1]*Department of Biomedical and Forensic Sciences, University of Cape Coast, Cape Coast, Ghana*

[2]*Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana*

[3]*University of Cape Coast Hospital, Ghana*

ARTICLE INFO

Article history:
Received 4 Dec 2014
Received in revised form 10 Dec, 2nd revised form 11 Dec, 3rd revised form 16 Dec 2014
Accepted 13 Mar 2015
Available online 21 May 2015

Keywords:
Schistosoma haematobium
Plasmodium berghei
Parasitaemia
Survivability

ABSTRACT

Objective: To investigate the effect of pre-existing *Schistosoma haematobium* (*S. haematobium*) infection on malaria disease severity.

Methods: The study involved the use of twenty-five imprinting control region mice, fifteen of which were initially infected with *S. haematobium*. Five of the remaining ten schisto-uninfected mice together with five schisto-infected mice were infected with *Plasmodium berghei* (*P. berghei*) after four weeks (acute stage) of schistosoma infection. The remaining five schisto-uninfected mice together with five schisto-infected mice were also infected with *P. berghei* after seven weeks (chronic stage) of schistosoma infection. The last five schisto-infected mice were used as control group. They were then monitored for changes in *P. berghei* parasitaemia on Days 3, 5, 7, 9 and 11 post-infection. Records on their survivability were also taken.

Results: The co-infected mice had significantly higher malaria parasitaemia, compared with the mono-infected mice during acute *S. haematobium* infection. In contrast, the co-infected mice had significantly lower malaria parasitaemia during chronic *S. haematobium* infection and a higher survival rate.

Conclusions: Co-infection of mice with *P. berghei* during acute *S. haematobium* infection resulted in rapid *P. berghei* development and increased malaria parasitaemia. However, the co-infection resulted in slower *P. berghei* development and decreased malaria parasitaemia with enhanced survivability of the mice during chronic *S. haematobium* infection. Therefore, pre-existing chronic *S. haematobium* infection may provide some protection to the host by reducing parasitaemia.

1. Introduction

Schistosomiasis and malaria are the world's two most important parasitic infections in terms of distribution, morbidity and mortality [1]. Schistosomiasis and malaria are among the parasitic infections that share common transmission areas in various tropical regions. Recent studies in vertebrates have indicated that interactions between co-infecting parasites can be pronounced and have important consequences for disease development, severity and transmission dynamics [2,3]. Therefore, there is the need to determine how a given parasite will interact with another in the same host. It will show whether the presence of one parasite in the host hinders the activities of the other.

Although interactions between helminthes and malaria parasites could affect both parties, research has mostly focused on the extent to which helminth co-infection influences the malarial disease. In the past few years, studies have been conducted to elucidate the immune mechanism(s) involved in worm and malaria co-infections [4,5]. However, many of these studies have produced conflicting results, which has made it difficult to clearly understand the outcomes of these co-infections [5–14]. Some studies have reported an increased incidence of

*Corresponding author: Benjamin Amoani, Department of Biomedical and Forensic Sciences, University of Cape Coast, Cape Coast, Ghana.
Tel: +233 244187291
E-mail: proffemu@yahoo.com

Peer review under responsibility of Hainan Medical University.
Foundation Project: This work was supported and funded by the Department of Biomedical and Forensic Sciences of university of Cape Coast, Ghana.

falciparum malaria in hosts with *Schistosoma mansoni* (*S. mansoni*) [6,10], while other studies have indicated that *Schistosoma haematobium* (*S. haematobium*) provides some protection from malaria such as lower parasitaemia and lower incidence [7–9].

Speculations on how helminthic infections may alter the susceptibility to clinical malaria have led to an increasing interest in investigating the consequences of co-infection. These studies have yielded contrasting results. Earlier studies reported a decreased malaria parasitaemia in co-infected mice whereas a recent one reported that mice with ova producing *S. mansoni* infection had increased malaria parasitaemia with *Plasmodium* infection [8,10–13].

Most studies that examined naturally occurring co-infection in humans indicated that co-infection with *schistosoma* and malaria parasites had an effect on the host, both in terms of pathological and immunological responses [14]. The direction of this response seems to depend on the host age, the malaria parasitaemia, the species of schistosome and the worm burden. Co-infection by these two parasites may have an important influence on the regulation of the immune response associated with the development of these infections and their respective morbidity [5,15,16].

Over the years, it has been more progressively speculated that helminth infections may change vulnerability to clinical malaria, and there is now escalating interest in investigating the repercussion of co-infection, with studies producing contrasting results [7–13]. Therefore, this study seeks to investigate the effect of pre-existing *S. haematobium* infection on malaria severity, using experimental animal model. Findings from our study will help to provide explanation to the observed trend of vulnerability or protection that pre-existing infection offers to new infections.

2. Materials and methods

2.1. Source of imprinting control region (ICR) mice

Male ICR mice, four weeks old, were purchased from the Noguchi Memorial Institute for medical research animal's house. They were kept in the animal facility at University of Cape Coast. Mice were maintained in ventilated cages and supplied with standard food and distilled water. The mice were randomly assigned to five groups, with each group containing five mice. The studies were conducted in accordance with accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC). Approval for this study was obtained from the Department of Biomedical and Forensic Sciences Ethical Committee.

2.2. Source of the helminthes and parasites inoculation

Infected *Bulinus truncatus* snails were collected from Baafikrom, a town near Mankessim, Central Region, Ghana. The snails were exposed to sunlight for about 30 min to shed their cercariae into clean water in Petri dish and checked for the presence and species of the cercariae using a dissecting microscope at 40× magnification to discriminate *S. haematobium* cercariae from bird and other animal schistosomes. Cercariae from different shedding dish (Petri dish) were pooled into test tubes and centrifuged at a low speed of 800 r/min (revolution per minute) using Centrifuge 5702 R to concentrate the cercariae and allow mixing of sexes to avoid the possibility of single infection. The sediments were then collected and used to infect the mice. About 50–100 cercariae were percutaneously injected into each mouse with 2 mL syringe, for three groups of mice (Groups 1, 2 and 3). Groups 4 and 5 mice were not infected with cercariae. Helminth infection was confirmed by the presence of worms by portal perfusion after four weeks [17].

Plasmodium berghei (*P. berghei*) was provided from Noguchi Memorial Institute for medical research (Legon, Ghana). Parasites were stored as frozen stabilates at −80°C. To obtain experimental inocula of *P. berghei*, packed red blood cells were passed through four donor mice. Infections were initiated in ICR mice by intraperitoneal injection of 0.2 mL of blood (packed red blood cells) containing 1.0×10^6 of *P. berghei* after four weeks of *S. haematobium* infection for Group 1 mice and Group 4 mice. After seven weeks of *S. haematobium* infection, Groups 2 and 5 mice were also inoculated with 1.0×10^6 of *P. berghei*. Group 3 mice served as control for the chronic infection. The various treatment groups were monitored for parasite development and growth in the mice [18].

2.3. Parasitaemia determination

Thin blood films from tail blood were made using standard microscope slides on Days 3, 5, 7, 9 and 11, air-dried and fixed in an absolute methanol for 5 min. The stained blood films were observed under a standard light microscope with 100× oil immersion lens. Infected and uninfected erythrocytes in different fields of view were identified and counted. Infected red blood cells were counted microscopically in at least five microscopic fields, each showing approximately 300 cells [10,17].

2.4. Mice survivability

Mice were monitored daily for mortality, to evaluate the survival rate of schistosome mono-infected, *P. berghei* mono-infected and the co-infected mice for both acute and chronic schistosome infection.

2.5. Statistical analysis

Differences in the parameters measured for the different test groups were tested for significance by the independent *t*-test using SPSS 17 computer software. Differences in between groups were considered significant when *P* value was less than 0.05. Percentage survivability were also used as comparing parameters for the different groups.

3. Results

3.1. Survivability

During the chronic co-infection, the *P. berghei* mono-infected group of mice survived until Day 5 after which they began to die on Day 6. Only 40% of them survived until Day 8 after which they also died before Day 11. On the other hand, the *Schistosoma–Plasmodium* co-infected group survived until Day 6 when they started to die. Eighty percent of them survived until Day 12 after which they all died by Day 14 (Figure 1). In