Journal of Scientific and Innovative Research 2013; 2 (3): 575-578
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2013; 2(3): 575-578

Received: 03-07-2013
Accepted: 17-08-2013

Anyaegbunam*
Deparment of Biological Science, Anambra State University Uli, Nigeria

Lucy Chinyere , Orakwelu
Deparment of Biological Science, Anambra State University Uli, Nigeria

Hodges Chinemerem, Obi, Zita Chika
Deparment of Biological Science, Anambra State University Uli, Nigeria

Correspondence:
Anyaegbunam
Deparment of Biological Science, Anambra State University Uli, Nigeria
Phone: +234-7062586150
E-mail:
lcanyaegbunam@yahoo.com

The evaluation of *Plasmodium falciparum* infection in Immune Suppressed Albino Mice

Anyaegbunam, Lucy Chinyere , Orakwelu, Hodges Chinemerem, Obi, Zita Chika

Abstract

Plasmodium species have been observed to be host specific as seen in *Plasmodium falciparum* which causes malaria in humans only. The establishment of *P. falciparum* malaria infection was evaluated outside human body through immune suppression in albino mice. 50 albino mice bought from Microbiology Laboratory of Anambra State University, Uli were used. The mice were divided into two groups A and B in the ratio 4:1 which served as test and control respectively. The test group was further divided into A1 and A2 (20, 20). Two phases of immune suppressions were employed; starvation and joint action of starvation and hydrocortisone. A confirmed *P. falciparum* infected human blood samples were collected by a Medical Laboratory Scientist into EDTA bottle which was used as source of *P. falciparum*. Group A1 mice were starved for 30hours and A2 for 50hours. The mice were infected with 0.1ml of the infected blood each and were starved for 3hrs before they were fed. The treated mice were observed for 3 weeks and were diagnosed using thick film microscopy. The results obtained had A1 (5.6%) positive with trophozoites and A2 (55%) positive. Further investigation followed 112 hours starvation in the previously treated mice. The A2 group was treated with 0.5ml hydrocortisone acetate and was observed for 3 days. Diagnosis showed 100% infection in both groups with parasitaemia ranging from 0.5 – 2.0% in A1 and 2.5 – 10% in A2. The duration of starvation and hydrocortisone affected the establishment of *P. falciparum* infection in experimental animal (mice). This work recommends a full study on mice immune system as well as erythrocyte surface protein to determine the actual factor responsible for the establishment of *P. falciparum* infection in mice.

Keywords: *Plasmodium falciparium*, Immunosuppression, Albino mice, Malaria, Starvation, Hydrocortisone.

Introduction

Malaria is caused by Protozoan parasites of the genus Plasmodium, five species of which are known to infect humans; *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowelsi*. *P. ovale* was recently proposed in persuasive molecular evidence to comprise two sub species[1] and *P. knowelsi*, a parasite of primate was able to infect humans through zoonosis.[2] The malaria parasite has a complex, multistage life cycle occurring within two living organisms, the vector mosquitoes and the vertebrate hosts.

The survival and development of the parasite within the invertebrate and vertebrate hosts, in intracellular and extracellular environment, is made possible by a tool knit of more than 5,000 genes and their specialized proteins help the parasite to invade and grow within multiple cell type and to evade host immune response.[3, 4]

Malaria parasite complex life cycle in their host involve clinical symptoms arising from cycles of erythrocyte invasion, growth and division followed by cell lysis and re-invasion. These stages of development such as the sporozoites (the infectious form injected by mosquito), merozoites (the stage that invade erythrocytes), trophozoites (the form multiplying in erythrocytes) and gametocytes (sexual stage) have their unique shapes and structures as well as protein complements. The surface proteins and metabolic pathways keep changing during these stages that help the parasite to evade the immune clearance, while creating problems for the development of drugs.[3]

Each species of Plasmodium has distinct physical (morphology) characteristics that are apparent under a microscope. In *P. falciparum*, only early ring-like trophozoites and gametocytes are seen in the peripheral blood. The parasitized erythrocytes are not enlarged and it is common to see cells with more than one parasite within them. *P. falciparum* is especially dangerous as this species is able to remodel the erythrocyte within 48hour asexual blood stage cycle, to enable infected cells to adhere to endothelial surface, thus progressively blocking microcapillaries, leading to obstructed microcirculation and consequent dysfunction of multiple organs, typically brain. In this way, the parasite reduces passage of infected erythrocytes through the spleen, which is able to detect and destroy such compromised cells.

Materials and Methods

In mice, certain strains of Plasmodia are known to cause malaria; these include *P. chabaudi*, *P. yoelic*, *P. berghei* and *P. vinckei*. In line with this, though P. falciparum is host specific, in this study attempts were made in evaluating the potency of starvation and hydrocortisone in establishing *P. falciparum* malaria infection in albino mice.

Experimental materials

10 weeks old mice both male and female bought from Microbiology Labouratory of Anambra State University, Uli were used. They were kept in an aerated metal cage and fed with granular poultry feed and clean water. The mice were classified into groups A and B; A1 (20), A2 (20) and B (10). Group A served as test group while B served as control.

Source of *P. falciparum*

Human whole blood sample infected by *P. falciparum* malaria parasite was collected on arrangement by a Medical Technologist with Amaechi Medical Laboratory Mgbidi, Imo State. The MP positive blood samples were kept in EDTA bottles and transported to the laboratory for use. The level of parasitaemia in the MP positive blood samples ranged from 5 – 10%, and was collected from patients that had presented clinical symptoms.

Preparation of Test Mice

The test mice were starved for 30 -50 hours prior to infection with 0.1ml *Plasmodium falciparum* positive blood sample through intraperitoneal route. The starvation period was extended for 3 hours post-infection after which they were fed.

Diagnosis and Identification

After 3 weeks post-infection, the mice were diagnosed for P. falciparum malaria parasite using blood films made from tail snip further treatments for immune suppression technique was employed.[5] This involved 112hrs for group A1 mice and 0.5ml hydrocortisone acetate at a concentration of 20mg/ml within the last 3 days of starvation for group A2 mice. On the 5th day, the mice were infected with fresh MP sample through intraperitoneal route. After the treatments, the starvation was extended for an hour before they were fed. There was massive death of the mice but those that survived were diagnosed for *P. falciparum* after five weeks post-infection.

Results

The results obtained in the different stages of treatments to establish infection in the experimental animal were as follows;

Table 1: Percentage parasite infection established in the test mice A1 and A2 after starvation; 30 and 50hrs respectively

Test Group	No infected and% infection	Non-infection and %
A_1	1(5.6%)	17(94.4%)
A_2	11(55%)	9(45%)

In the table, A_1 mice had 5.6% infection while A_2 had 55% infection.

Table 2: Percentage Parasitaemia in the tested mice A_1 and A_2 groups

% Parasitaemia	Group A_1	Group A_2
0.5	1	6
1.0	-	1
1.5	-	1
2.0	-	2
2.5	-	1
Total	1	11

The level of parasitaemia was highest in A_2 at 0.5% involving 6 mice while A_1 had only one mouse affected.

In the second phase of treatment, which involved hyrocortisone and starvation, the following results were obtained.

Table 3: Percentage parasite infection established in the test mice A_1 and A_2

Test groups	No infected and % infection	Uninfected and their %
A_1	7(100%)	Nil
A_2	9(100%)	Nil

In the table, 100% infection was observed in both groups (A_1 and A_2).

Table 4: The level of parasitaemia in the infected mice

% Parasitaemia	Group A_1	GroupA_2
0.5	3	-
1.0	2	-
2.0	2	-
2.5	-	2
3.5	-	4
4.5	-	2
10.0	-	1
Total	7	9

The level of parasitaemia in A_1 is between 0.5 – 2.0%, while A_2 is 2.5 – 10%.

Discussion

The results obtained confirmed the potency of hydrocortisone and/ or starvation in compromising mice immune system so as to establish *P. falciparum* infection. The independent effect of starvation showed significant difference in duration relative to parasite establishment and percentage parasitaemia in group A1. This is in line with the adverse effect of 48hour starvation on mice immune system. The immune suppression achieved was as a result of low blood leptin level, a hormonal association with T-cell production. A significant difference in parasitaemia observed was among the test mice in relation to the test condition. Increased parasite establishment and percentage parasitaemia within group A1 tends to further support the effectiveness of duration of starvation on ensuring such conditions. In group A2 mice, there was increased parasitaemia as well, ranging from 2.5 – 10.0%. This however, points to a more potent effect of combination of starvation and hydrocortisone relative to independent effect of starvation. Though cortisone has been implicated to be of sole effect on T-cells as well, the significant difference may be as a result of its ability to deplete T-cells at a higher rate or the synergistic activity of both starvation and hydrocortisone. This piece of work creates room for further investigation into the relationship between immune system of vertebrate host and *P. falciparum* infection in terms of drug resistance and the pathological effect associated with this.

Conclusion

Plasmodium falciparum, though host specific can be cultivated in other vertebrate host by immune suppression. The establishment of *P. falciparum* infection in Albino mice gives leverage in the use of laboratory animal to investigate the factors responsible for the resistance of this species of plasmodium to malaria drugs.

Reference

1. Sutherland C.J., Tanomsing N. and Nolder D. Two nonrecombining sympatric forms of the Human Malaria parasite Plasmodium Ovale occur globally. Journal of infectious Diseases. 2010; 201(10):1544-1550.

2. White N.J. Plasmodium Knowelsi: The fifth Human malaria parasite. Clinical Infectious Diseases 2008;46 (2): 172-173.

3. Brain M.G., David A.F., Dennis E.K., Stefan A.I., Pedro. L.A., Frank H.C., Patrick E. D . Malaria Progress: Perils and prospects for eradication. Journal of clinical Investigation, 2008; 118:1266-1276.

4. Laurence F., Michael P.W., Dale J.R. A proteomic view of the plasmodium life cycle. Nature. 2002; 419: 520-526.

5. Ludovic A., Rajeer K.T., Pedro M., Nico V.R., Jean-Louis P. Pierre D. Analysis of innate defences against Plasmodium falciparum in immunodeficient mice. Malaria Journal. 2010; 9:197.