Effect of Chloroquine Sensitive *Plasmodium berghei* in Pregnant Mice

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**Abstract:** Pregnant mice were examined to determine whether or not they transmitted *Plasmodium berghei* to their fetuses. On the 14th day of pregnancy, mice were inoculated with approximately 3×10⁴ *P. berghei* infected red blood cells by intraperitoneal injection. The parasitemia in 20 adult females and 145 neonates was assessed using thin blood films fixed with methanol and stained with 10% giemsa solution. The average parasitemia of females at delivery was 7.5%. Malaria parasites were microscopically confirmed in 8 of the 145 neonates. Maternal parasitemia at the time of delivery was not correlated with the incidence of vertical infection (8.71%). Present study showed that this model may be used to examine vertical transmission of malaria.

**Key words:** Congenital malaria, *Plasmodium berghei*, pregnant mice, neonates

**INTRODUCTION**

Malaria is a mosquito borne disease caused by *Plasmodium* sp. Only the malaria type caused by *Plasmodium falciparum* is known life threatening. Infection with *P. falciparum* is therefore a medical emergency (Hardman and Limbird, 2001). Malaria is a major health problem in Nigeria and one of the most prevalent diseases in the world. Every year, thousands of cases are reported from all over the country (Martens and Hall, 2000).

Malaria continues to be a major cause of morbidity and mortality in tropical countries. About 2% of persons infected with *falciparum* malaria die, usually because of delayed treatment (William et al., 2004). The reality is probably worse than traditionally cited figures: recent analysis estimates, at a minimum, between 700,000 and 2.7 million deaths each year from malaria (>75% of them African children) and between 400 and 900 million acute febrile episodes per year in African children under the age of five living in malaria endemic regions (Breman et al., 2001).

During pregnancy-associated malaria, *Plasmodium falciparum* infected red blood cells are sequestered in the placenta causing low-birth-weight, foetal and infant death and material anaemia (Craig, 2004; Guyatt and Snow, 2001).

In the placenta, adhesion seems to occur between the host receptor Chondroitin Sulphate A (CSA) and *P. falciparum* erythrocyte membrane protein one (PFEMP1) (Buffet et al., 1999; Fried and Duffy, 1996; Gamain et al., 2004). Congenital malaria is said to increase the mortality of children under 5 years of age. This form of malaria is defined as the presence of parasitemia in the peripheral blood of neonates <7 days of age, thereby excluding the possibility of having acquired the infection from sporozoites (Loke, 1982).
Akindele et al. (1993) reported an incidence of peripheral parasitemia in neonates of more than 20% and more recently, Fischer (1997) reported variability in the prevalence from undetectable to 23%, with a median of 7%. These large variations in the purported prevalence of congenital malaria cannot be explained by biased selection, seasonal factors or antenatal prophylaxis. Efforts have been made to establish an animal model for congenital malaria.

Brace-Chwatt and Gibson (1955) first reported that mice and rats rarely transmitted parasites to their fetuses. Later, Gillet and Herman (1974) indicated that 51 of 52 embryos and 41 of 48 new born mice had parasitemia, however other researchers did not find such a high incidence of congenital malaria in rodents (Oduola et al., 1982; Desowitz et al., 1989). Fischer (1996) found no evidence for the vertical transmission of malaria in Wistar rats. Das Gupta (1934) was unable to demonstrate vertical transmission in a rhesus monkey (Macaca mulatta), whereas Davison et al. (1998) documented congenital infection with Plasmodium coatneyi in 1 of 7 monkeys.

In recent time, much detailed study had not been conducted in the area of congenital malaria using mice model. In the present study, we investigated the effect of chloroquine sensitive Plasmodium berghei in pregnant mice.

MATERIALS AND METHODS

Fourteen-week-old pregnant mice were used for this study after 12 days of conception. The female mice had not been pregnant before. The pregnant mice were infected with Plasmodium berghei ANKA strain. Infected mouse erythrocytes were diluted with Phosphate Buffer Saline (PBS) so that approximately $3 \times 10^6$ parasites were injected intraperitoneally into 20 pregnant mice at day 14 of gestation. Control mice of the same strain that were not pregnant were identically inoculated to follow the course of maternal infection.

The parasitemia was assessed from the blood collected from the tail vein of pregnant mice on the day of delivery and from control females on the same day. Thin blood smearsfilms were fixed with methanol and stained with 10% Giemsa solution. Four days after delivery, neonatal blood films were prepared, fixed and stained as earlier described. The stained glass slides were viewed under a high-powered microscope using x100.

This study was carried out at the Nigerian Institute of Medical Research, Maiduguri Outstation, Maiduguri, Nigeria from June to August, 2006.

RESULTS AND DISCUSSION

Twenty pregnant mice and their 145 neonates were examined. Seven out of 20 females transmitted parasites to their fetuses (Table 1). Not all pups from a female were infected. Only a small proportion of pups from the same female was infected. The average parasitemia of these females at the time of delivery was 8.71% ranging from 3.5 to 20.5%. The remaining 13 females delivered 102 pups and there was no vertical infection. The average parasitemia of these females was 6.85% ranging from 1.5 to 22.5%. There was no significant difference between the parasitemia of females that transmitted parasites to their fetuses and those that did not.

To check parasitemia in the neonates, 70 pups were examined on day 0, 30 on day 1, 20 on day 2, 15 on day 3 and 10 on day 4. These division was necessitated in order to avoid unambiguous results and tediousness. Eight samples were found to be positive. Therefore, in this study, 8 of the 145 neonates were congenitally infected, a prevalence of 5.5%.

Malaria in pregnancy is unique in some aspects. It is well known in both humans and mice that vertical transmission of infection from mother to child is rather rare, although the placenta heavily sequesters parasitized erythrocytes. In mice, maternal infection by Plasmodium during pregnancy
Table 1: Vertical transmission of *Plasmodium berghei* malaria to neonate mice

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<th>Dam’s percentage parasitemia at delivery (%)</th>
<th>No. of tested</th>
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<td>20.5</td>
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Average 7.5%; Total 145, 8 % Positive (Neonates) 5.5%

often results in a high concentration of parasitized erythrocytes in the maternal intervillous space and causes Massive Chronic Intervillositis (MCI) of the placenta (Labarrere and Mullen, 1987; Tegoshi et al., 1992). In humans the intervillous space is also filled with parasitized erythrocytes and inflammatory cells, e.g., monocytes and macrophages, some polymorphonuclear leukocytes and scattered T and B lymphocytes (Ordi et al., 1998). Tegoshi et al. (1992) compared placental histology of malarial-infected humans and rats. Although there were minor differences, the major pathological changes, e.g., thickening and duplication of the trophoblastic basement membrane, were the same.

The mouse placenta is more similar to that of a human than that of carnivores and ruminants. Primates and rodents have characteristic placentas that do not have maternal tissues and the fetal chorion is bathed directly by maternal blood through the maternal intervillous space. However, the number of trophoblast layers differs between humans and rodents. Rodents are hemochorial, whereas humans are hemomonochorial.

In the human placenta, only the syncytiotrophoblast hampers malaria transmission from mother to child in the placenta (Loke, 1982). Nevertheless, placental infection is normally confined to the maternal space and is absent in the fetal erythrocytes in the villi (Miller and Smith, 1998). Pavia (1983) showed that the monolayers of mouse trophoblast phagocytosed a significant portion of malarial by-products during infection with *P. berghei*. Davison et al. (1998) reported that the rhesus monkey was a good animal model in terms of the malarial symptoms that appeared during pregnancy. Although it is obvious that monkey is a better model than a mouse, the management of monkeys is difficult and costly. The combination of mouse and *Plasmodium berghei* may be a suitable alternative model for congenital malaria.

**CONCLUSIONS**

Since this mouse model is less difficult and cheaper, it may be used to study and examine vertical transmission of malaria.
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REFERENCES


