



Original article

Susceptibility of mice strains to oxidative stress and neurotransmitter activity induced by *Plasmodium berghei*Esam M. Al-Shaebi^a, Walid F. Mohamed^b, Saleh Al-Quraishy^a, Mohamed A. Dkhil^{a,c,*}^a Department of Zoology, College of Science, King Saud University, Saudi Arabia^b Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Egypt^c Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt

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ABSTRACT

This study investigated the susceptibility of female C57Bl/6 and Swiss Albino mice to oxidative stress and neurotransmitters activity induced by *Plasmodium berghei*. On day 9 p.i. with *P. berghei* infected erythrocytes, the mice reduced in weight. This weight loss was markedly higher in SW mice and reached about –14%. Also, the infection was able to cause oxidative damage to the brain tissue. Catalase activity as well as glutathione, malondialdehyde and nitric oxide levels were different in the two mice strains. Moreover, the brain content of neurotransmitters, epinephrine, norepinephrine, dopamine and serotonin in mice brain was higher in SW mice than B6 mice. We concluded that, the strain of mice is one factor that could alter the response of mice to *P. berghei* infection.

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1. Introduction

Cerebral malaria (CM) remains a very dangerous complication of infection causing a high mortality rate (Mehlhorn, 2014). According to the latest world health organization report, there were 214 million cases of malaria in 2015 and 438,000 deaths (WHO, 2015). In human, CM is due to *Plasmodium falciparum* or *Plasmodium vivax* infection and cause several neurodegenerative diseases (Apoorv and Babu, 2016).

Plasmodium berghei infection of mice is a widely used model of experimental cerebral malaria (Martins et al., 2016). Concerning the oxidative damage and changes in brain content of neurotransmitters, there is no available information about the strain difference effect on susceptibility of mice to *P. berghei* infection. However, there is a clear change in sex difference to susceptibility of mice to *P. berghei* infection (Dkhil et al., 2016).

Scheller et al. (1994) studied the susceptibility of different strains of mice to hepatic infection with *P. berghei*. Also, Randall

et al. (2008) reported a significant heterogeneity between CBA/CaH and C57BL/6 mice infected with *P. berghei*.

The current study aimed to investigate the oxidative damage and neurotransmitters activity induced by *Plasmodium berghei* in C57Bl/6 and Swiss Albino mice.

2. Materials and methods

2.1. Mice strains

Both of Adult females C57BL/6 and Swiss albino mice were obtained from the animal facility of King Faisal hospital at Riyadh. Animals were maintained in a specific pathogen-free condition at the Department of Zoology animal housing facilities in strict accordance with the institutional and national official guideline for the project number RG-198. *Plasmodium berghei* were passaged in mice and just as parasitaemia reached about 20%, parasitized blood was taken to infect C57BL/6 (B6) and Swiss albino (SW) female mice. All infected mice received an intraperitoneal injection of 1×10^6 *P. berghei*-infected erythrocytes. Parasitemia was calculated in blood smears stained with Giemsa. Cell number was estimated using a Neubaer-chamber.

2.2. Tissue preparation

Twelve mice of each strain were sacrificed by cervical decapitated on day 9 Postinfection (p.i.). Brains were rapidly excised from

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skulls; weighed then stored at -80°C for biochemical studies. In each group; six brains were used for the histological study and each brain of other 6 mice were divided into two halves. The first half was used for oxidative stress experiment and the second half was used for determination of neurotransmitters contents.

2.3. Oxidative stress biomarkers

According to Tsakiris et al. (2004), the isolated brain tissues were homogenized in ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose, pH 7.4. This brain homogenate was used for biochemical investigations.

Brain glutathione (GSH) level was estimated by the method of Ellman (1959). The method depends on the reduction of Ellman's reagent with GSH to give a yellow compound; the reduced chromogen directly proportional to GSH concentration. The absorbance was measured at 405 nm.

The level of nitric oxide was determined according to the method of Green et al. (1982). Briefly, in an acid medium and in the presence of nitrite the formed nitrous acid diazotise sulphanilamide is coupled with *N*-(1-naphthyl)ethylenediamine. The formed azo dye contained a bright reddish-purple color was measured at 540 nm.

Malondialdehyde level was determined according to the method of Ohkawa et al. (1979) by using 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67% and were then heated in a boiling water bath for 30 min. Thiobarbituric acid reactive substances were determined by the absorbance at 535 nm.

The activity of catalase in brain homogenate was estimated by the method of Aebi (1984). In this assay, catalase combines with a known quantity of H_2O_2 and the reaction is stopped after exactly one minute with a catalase inhibitor. In the existence of horseradish peroxidase, the remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to give a chromophore with a color intensity inversely proportional to the extent of catalase in the original sample, and then determined at 240 nm.

2.4. Estimation of neurotransmitters contents

The content of epinephrine, norepinephrine, dopamine and serotonin was determined according to the method of Ciarlone (1978).

2.5. Statistical analysis

Statistical analysis was achieved by using an unpaired Student's *t* test. MS Excel 2007 (Microsoft, Rochester, NY, USA) and SigmaPlot 2011 (Systat Software, Inc, Chicago, IL, USA) were used for data analysis.

3. Results

P. berghei infection induced a significant difference ($P \leq 0.01$) in parasitemia between B6 and SW mice (Fig. 1). This clear significant difference with increased parasitemia in SW mice was detected on days 5–9 p.i. (Fig. 1).

On day 9 p.i. with *P. berghei* infected erythrocytes, the mice reduced in weight. This weight loss was markedly higher in SW mice and reached about -14% (Fig. 2).

Histological alterations were also observed between the two mice strains. In SW mice, there were more blood appeared in the haemorrhage area. Also, some more neural cells appeared vacuolated. Moreover, the Purkinje cells were destructed in SW mice more than B6 mice (Fig. 3).

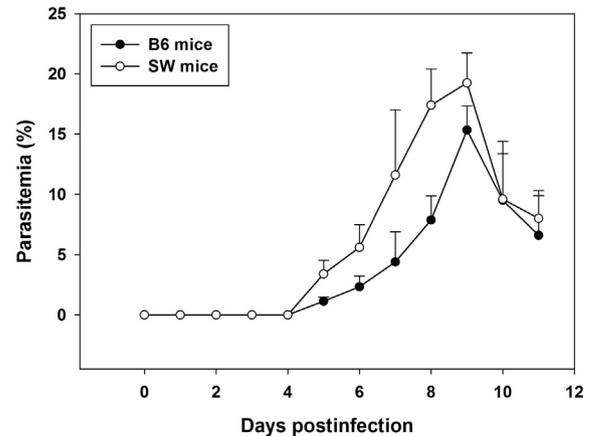


Fig. 1. Parasitemia of B6 and SW mice infected with *P. berghei*. Values are means \pm SD.

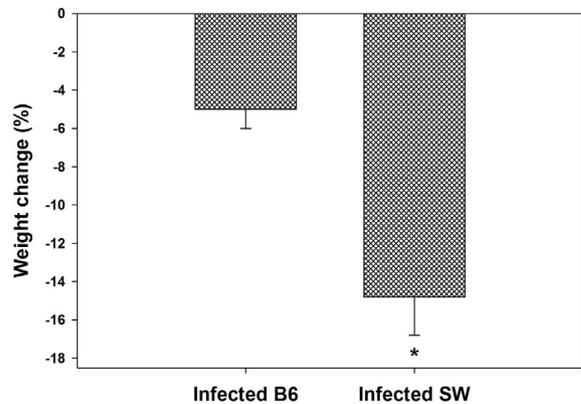


Fig. 2. Weight change in mice at day 9 p.i. with *P. berghei*. Values are means \pm SD. *Significant change at $P < 0.01$ between B6 and SW mice.

The infection was able to cause oxidative damage to the brain tissue. Catalase activity as well as glutathione, malondialdehyde and nitrite/nitrate levels were different in the two mice strains (Table 1).

A change in the brain content of neurotransmitters was clearly observed through the significant alteration in epinephrine, norepinephrine, dopamine and serotonin. The brain content of these neurotransmitters in mice brain was higher in SW mice than B6 mice (Table 2).

4. Discussion

C57Bl/6 mice were found to be more susceptible to *P. berghei* infection than Swiss albino mice. Strain specificity of the disease depend on genetically determined physiological factors as the rate of parasite proliferation (Brewer and Powell, 1965) or host's immune responses, restricting parasite multiplication or producing auto-antibody and immunological injury (Voller, 1974; Mackey et al., 1980). Such strain specific factors which may be reflected by changes in the haemogram, organ weight or structural and functional lesions of organ systems can be compared with those in other animals and in human malaria (Sadun et al., 1966). In this study, the parasitemia, mice weight and the histopathological lesions in brains of B6 and SW mice were significantly different. In general, the infection induced weight loss due to the disturbances in the mice metabolism and the loss of mice appetite (Dkhil et al., 2016).

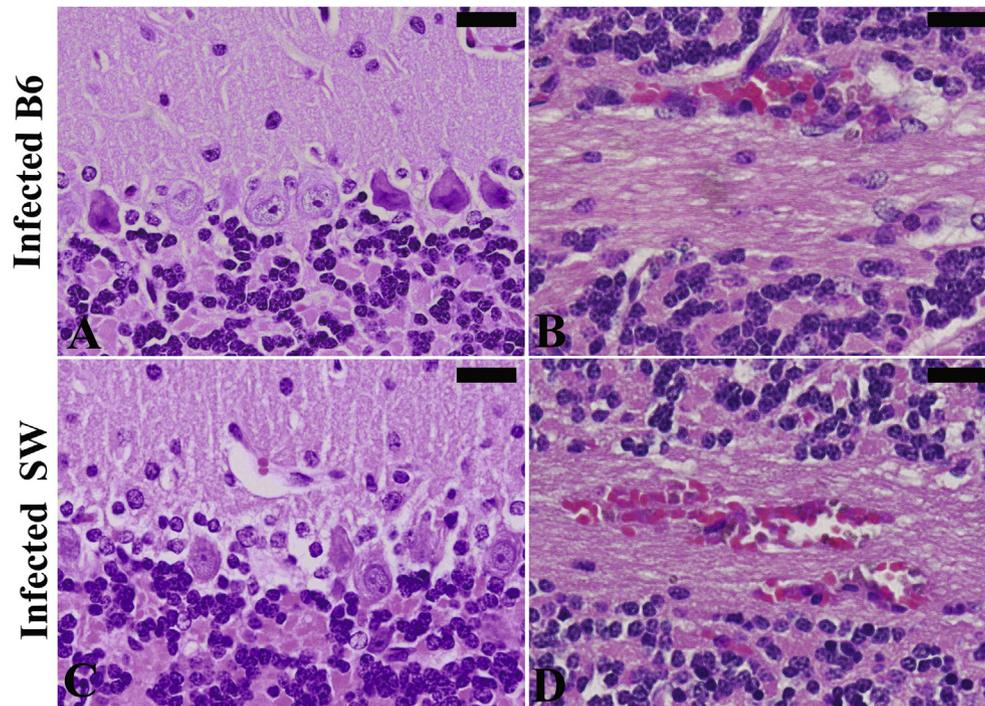


Fig. 3. Histology of mice brain infected with *P. berghei*. (A, B): Infected B6 mice. (C, D): Infected SW mice. Sections are stained with hematoxylin and eosin. Scale bar = 25 μ m.

Table 1

P. berghei Infection induced changes in the activity of catalase and the level of glutathione, malondialdehyde and nitric oxide.

Parameter	C57Bl/6 mice	Swiss albino mice
Catalase (U/g)	2 \pm 0.3	1.8 \pm 0.1*
Glutathione (mg/g)	9.7 \pm 4	6.3 \pm 0.3*
Malondialdehyde (nmol/g)	99 \pm 1.4	102 \pm 1
Nitric oxide (μ mol/g)	243 \pm 9	210 \pm 3*

Values are means \pm SD.

* Significant change at $P < 0.01$ between B6 and SW mice.

Table 2

P. berghei infection induced changes in brain content of epinephrine, norepinephrine, dopamine and serotonin.

Parameter	C57Bl/6 mice	Swiss albino mice
Epinephrine (μ g/g)	355.5 \pm 4.5	555.5 \pm 4.2*
Norepinephrine (μ g/g)	311.4 \pm 4.24	572.8 \pm 6.3*
Dopamine (μ g/g)	901 \pm 7.6	1511.74 \pm 5.7*
Serotonin (μ g/g)	179.43 \pm 5.8	323.6 \pm 3.13*

Values are means \pm SD.

* Significant change at $P < 0.01$ between B6 and SW mice.

Neurotransmitters are chemicals found in nerve cell link area with another cell at synapse, for signaling regulation (Mele et al., 2010). In general, some parasitic infection lead to a change in neurotransmitters such as *Toxoplasma* (Gatkowska et al., 2013), *Schistosoma mansoni* (Bauomy et al., 2013), *Toxocara canis* and *Trichinella spiralis* (Abdel Ghafar et al., 1996).

It was suggested that the production of reactive oxygen species is associated with oxidative stress and could plays an important role in the formation of the complications caused by malaria (Percário et al., 2012). The induced oxidative stress cause changes in erythrocytes and endothelial cells and facilitating the penetration of plasmodium in brain tissues (Kumar and Bandyopadhyay, 2005)

In this study, the difference in mice strain susceptibility to infection is related to the difference in response of mice glutathione, catalase, malondialdehyde and nitric oxide.

Clark et al. (1992) postulated that, pathogenesis of cerebral malaria is due to the increase in nitric oxide, which in turn leads to cerebral coma due to a difference in the neurotransmitters (Taylor-Robinson, 2010). In the current study, we observed an increase in the measured, dopamine, epinephrine, norepinephrine and serotonin. This increase in the parameters may help to reduce the body temperature, which reduces the risk of malaria (Dascombe and Sidara, 1994).

Dascombe and Sidara, 1994, demonstrated that an increase in the level of serotonin during infection with *P. berghei* may help to the host's overcome risk of fever. Also, Roy et al. (1993) reported a decrease in some neurotransmitters such as serotonin and norepinephrine and no change in histamine and dopamine in Albino mice and Albino rats during infection with *P. berghei*.

Based on our results, we can conclude that the strain of mice is one factor that could alter the response of mice to *P. berghei* infection.

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