Evaluation of the malaria development in a group of CB6F1 mice

Abstract

Purpose: The purpose of this assay was to characterize the infection by P. berghei in three groups of inbred mice produced in the facilities laboratory animals of the INDICASAT-Panamá, to determine the group more convenient to use as a biomodel in ethnobotanical compound testing against malaria.

Materials and methods: A group of mice CB6F1 (BALB/c x C57BL/6) and two inbred strain of mice: C57BL/6 and BALB/c, were parasitized with P. berghei (ANKA), and were evaluated the weight average, parameters of behavior and the integrity of Brain Blood Barrier, these data were related to the parasitaemia. The behavior parameters assessment allowed evaluate both general health and neurological reflexes of the mice and these were correlated with four stages involved with the pathology of the Cerebral Malaria.

Results: In reference to the average weight, all the animals of the three groups lost weight, this decrease did not present significant differences between them (p=0.8841, p>0.05). When relating the four stage with the developmental of malaria, the stages III and IV were related to the manifestation of Cerebral Malaria, verified by the Brain Blood Barrier injured. Thirty seven percentage (37%), of the CB6F1 mice showed signs of Stage IV, sixty-three percentage (63%), showed signs of Stage III, both with 24% of parasitaemia. The 100% of the C57BL/6 mice presented the Stage III, with 28% of parasitaemia, and the BALB/c didn’t presented cerebral malarial signs, although presented parasitaemias of 60%.

Conclusions: The tests carried out allowed relate functional and neurological behavior patterns with percentages of parasitaemia, and determine to the CB6F1 mice as a model that manifests Cerebral Malaria with low percentages of parasitaemia, as occurs in humans, considered susceptible mice. At the same time, that was verify the condition of susceptible strains and non-susceptible strains to BALB/c, C57BL/6, the mice reproduced in the INDICASAT AIP Bioterio, for to realize assays of antimalarial etnobotanicals agents.

Keywords: mice, cerebral malaria, C57BL/6, BALB/c, CB6F1, P. berghei, public health, malaria disease, patients, neurological deficits

Introduction

The infection of the malaria is caused by protozoa parasites of the genus Plasmodium. In accordance with the latest inform of the World Health Organization, it is of the main global public health problem. More than 212 million of new cases per year, being responsible of the death of 429,000 affected; it also reported in this inform that 91 countries were endemics, including American continent countries with the exception of USA, Costa Rica and Argentina.1

Cerebral malaria (CM) is the main fatal complication in the malaria disease, and it affects mainly children under the age of 5 years in sub-Saharan Africa [12]. The early diagnosis of the CM it is not generally successful, and even with the available treatment the 15–20% of the patients died, while 10–15% of the cured patients will suffer long-term neurological deficits.2,3

Experimental malaria studies have been carried out in different murine models, studies as physiopathological, molecular mechanisms and pharmaceutical products and others.4,5 Another studies on developmental phases of the parasite and clinical manifestation, have allowed qualify the mice as susceptible and no susceptible models.

The susceptibility condition is in relation with cerebral malaria pathology.6

The clinical signs in the susceptible mice when the cerebral malaria pathology is present are: ataxia, respiratory distress, development of neurological signs; coma and death, and they occur to the 8-10 days post infection. The histopathological analysis reveal vascular clogging, petechial hemorrhages and leukocyte sequestration with a relatively low sequestration of infected red blood cells.7,8

Different neurological malaria cerebral signs that occur in the humans are difficult to identify in the mice,9 analyses based on the time of infection may give origin to bias, derived of the different stages that happens in the disease in the animals.10 In the mice the neurological signs (ataxia, convulsions, roll over, paralysis, coma) are developed only few hours before death.11 Moreover, different factors as: background genetic related, age, inoculum size, parasitaemia course and clonal variations of the parasite also interfere with the incidence of CM in mice,12 for this reason is important the evaluation to identify the different stages of the disease and its manifestations, for characterize the infection in the mice on different strains.
Differents authors had used the protocol SHIRPA (SmithKline Beecham, Harwell, Imperial College, Royal London Hospital), the protocol evaluate the behavior and the development of neurological signs in the mice, comparing the behavior of parasitized mice with Plasmodium, and to relate it to the neurological signs that occur in CM, using this protocol is have characterized and presented differences between strains of mice.15,16

Similarly, the cerebral oedema pathology is implicated in the MC, explaining the permeability changes in the Brain Blood Barrier (BBB) in susceptible mice.17 The Evans Blue solution has been used, for evaluated the BBB injury and this way the clinic pathology of the disease in the mice, by the intensity of the blue coloration in the cerebral tissue. This is possible by bind with the plasma albumin that quickly across the BBB.18

The objective of the study was evaluated and compared some phenotypic manifestations (weight average) and some neurology signs implicated in the MC, and related with parasitaemia and BBB integrity, in mice CB6F1 mice groups and compare its, with C57BL/6 mice characterized as susceptible and with BALB/c mice characterized as non susceptible, when they are parasitized with P. berghei (ANKA). For have murine models characterized and so evaluate antimalarial etnotropic compounds, in the INDICASAT AIP-Institute of Panama.

Materials and methods
Animal model and malaria infection

Seventy (60) female mice were used, twenty (20) mice by three different genetic groups: C57BL/6, BALB/c and CB6F1. Two assays group were formed: one (1) parasitized group and one healthy mice control group. The mice were 7 to 8 week old. Animals were housed in conventional facilities at the INDICASAT AIP. These were housed in ventilated racks and received standar sterilized diet (LabDiet, USA) and water ad libitum, with sanitary barriers; sterilization of swarf and food, filtered water and change of clothes of the personnel. In room with 12 hours light: 12 dark hours.

Mice were infected with *Plasmodium berghei* (donated by Patricia Llanes PhD, of the Biology Cellular and Molecular of the Diseases Center. INDICASAT AIP), with intraperitoneal injection of 5x10⁶ parasitized red blood cells (pRBC). The pRBC were isolated from the blood of infected BALB/c mice with 30 to 40% parasitaemia (5x10⁶ parasitized cells), obtained of intracardiac blood in anesthetized mice with ketamine/xylacine 120/10 mg/kg weight. The control group was injected with an equivalent volume of healthy erythrocytes from the same mouse strain.

Research complied with all relevant guidelines and institutional policies of the INDICASAT AIP for the laboratory animal use and the approval of the CICUA-16-009.

Evaluation of clinical parameters

1. The weight average was registered using a Lab balance Model Scout II, OHAUS, accuracy 600x 0,1g. The weighing was realized daily among 8:00-10:00 am.

2. The developmental of the infection was evaluated daily with slide stained with giemsa (marca Merck) of the peripheral blood and counting the infected erythrocytes in 1000 cells RBCs, with light microscope to 100X, from first day onwards.

3. Eight (8) parameters were used for evaluated of the evolution of the infection with *Plasmodium berghei*, allowing determine the healthy condition and the neurological reflexes of the mice. The criterion used in the evaluation consisted in give score to the behavioral of the animals. The highest scores (3), indicated a healthy condition and normal neurological reflexes, simillary to control animals. Medium scores (2–1) indicated deteriored health condition and the deteriored neurological reflexes, and lowest (0) scores indicated condition bad of health and the neurological reflexes bad. Other criterions were evaluated as present (1) or absent (0). The percentages of the parasitaemia were related with some behavioral phenotypic parameters, used in the SHIRPA protocols.14,20,21 Other parameter evaluated was the permeability of the Blood-Brain Barrier (BBB).11

The parameters evaluated were measure daily. The behavioral parameters were measured placing to the mice in open field away from the accommodation cage, by a period of time of 5 minutes / animal / day, the times were determined using a stopwatch. The open field was permanently cleaned with alcohol - 5% water. The parameters evaluated and the scores used are presented in Table 1.

| Table 1 Behavioral parameters and scores for determine Cerebral Malaria |
|---------------------------|-----------------|-----------------|-----------------|-----------------|
| **Behavioral parameters** | **Score**       | **2**           | **1**           | **0**           |
| Locomotor activity        | walk quickly    | walk slowly     | walk and stop   | Does no walk    |
| Straightening reflex*      | return immediately | after of 2 attempts | after of 5 attempts | remains on the back |
| Pelvic elevation           | -               | on its 4 tips   | of side         | markedly fainted |
| Elevated tail              | -               | elevated        | little elevated | flattened       |
| Exhaust reaction to finger pressure | - | response | attempts response | no response |
| Tremor                     | -               | -               | absent          | present         |
| Piloerection               | -               | -               | absent          | present         |
| Deviation of head          | -               | -               | absent          | present         |

The behavioral parameters were relationated with alterations neurological associated with Cerebral Malaria and compared among parasitized mice and healthy mice. The Cerebral Malaria signs were presented in four stages, similar to propose Martinez et al.,13 as: Stage

Citation: Jesus RD, Spadadora C. Evaluation of the malaria development in a group of CB6F1 mice. MOJ Bioequiv Availab. 2018;5(5):264–268. DOI: 10.15406/mojbb.2018.05.00112
I (asymptomatic), in which parasitized mice have a behavior similar to mice healthy (controls), presenting high scores of 16, and without neurological alterations. The Stage II, in which the mice begin to observe neurological alterations, the score of the parameters evaluated was lower of 16. In the Stage III, the mice presented score lower of 12 and in the Stage IV, the clinic signs of cerebral malaria are evident the behavior parameters presented scores of zero (0), in all evaluated parameters.

The Brain Blood Barrier (BBB), was evaluated when the parasitaemia was of 24% for all the mice of CB6F1 and C57BL/6 strain and of 60% in mice of BALB/c. To the mice was administered 200μL by intraperitoneally way of solution Blue Evans to 2%, one (1) hour after, the animals were sacrificed with a ketamina/xilacyna mixture (brand: KEPRO–HOLLAND); to a dosage of 120/10mg/kg of weight, the brain was observed and the blue coloration grade was determinate.

The parameters evaluated of the SHIRPA protocol and the BBB influence as a method of evaluating the evolution of cerebral malaria with P. berghei in three groups of mice produced in the INDICASAT AIP facilities, were selected by your confiability and reproducibility high quality, requiring few equipmet and being a economic option.

Statistic analysis

The percentages of parasitaemia and average of the weight were expressed as means and standard errors of the mean (SEM). The normality of the dates was made by the Shapiro-Wilk test with a significance of P>0.05. The comparison between dated was made by Kruskal-Wallis One Way AOV - AOV Table (Statistix 10 Programmed) to the observations of the 7 days of the infection.

Results and discussion

The murine models BALB/c and C57BL/6, have been used in malaria assays, and they are susceptible and no susceptible, respectively, depending on the ability to suffer cerebral malaria, however, the neurological signs in the incidence of the cerebral malaria in the murine models present contradictions, either by the genetic background, age and inoculate size, the infection course of the parasite, among others.

In the assay realized the parasitaemia evolution since the first day post inoculation presented among the three groups of mice used: C57BL/6, CB6F1 y BALB/c. The C57BL/6 and CB6F1 mice presented a parasitaemia with a constant increases and continuous, across the observation time, and to day seven reached a percentage of 28±2,3% y de 24±1,5%, respectively. While the mice BALB/c presented a evolution with increases and decreases of the parasitaemias percentages across of the 11 days, with a average of 26,5±2,1% (Figure 1). The parasitaemia statistic analysis to the seven days, no presented significant differences with a P=0,895 (p>0,05).

Results similars were reported in assays with C57BL/6 mice, however, others reports presented parasitaemias highest of 80% for this strain to the 14 days post infection. And in relation to BALB/c mice parasitaemia, it has been reported different results were found encountered, in relation to increases and decreases along of the infection, but have been reported conditions of hyperparasitaemias.

In relation to weight average, all the mice lost weight. The mice C57BL/6 lost a average of -2,90%, the mice CB6F1 a average of -2,62% and the mice of the strain BALB/c -1,46%, respectively, and no presented significant differences with a p=0,8841 (P>0,05).

similarly, were presented. The mice of the group CB6F1 presented weight and parasitaemia percentages, with values intermediates, in relation to the others groups of the assay. In behavioral studies, groups similars of mice presented intermediates behavioral to other mice of the study.

The Figure 2, present some images that was observed in the three groups of mice during parasitaemia development, the behavioral parameters here studied they were relacioned with Cerebral Malaria.

The images a y b present the behavioral of the BALB/c mice group, in the (a) image, is observed a mice with one day post inoculated and parasitaemia 1%, the behavioral was similar to control. The image b, present a BALB/c mice with eleven (11) days post inoculated, with parasitaemia of 60%, and the phenotypic characteristics are: donot have locomotor activity (score 2), they have piloerection (P) and flattened tail (score 0), but does not presented Cerebral Malaria signs, and in the evaluation of the Blood Brain Barrier there not had injure (Imagenes 2a, 2b), therefore, they no suffered Cerebral Malaria despite the high parasitaemias.

In the Figure 2, it possible observe the behavioral of the C57BL/6 mice, in the image 2c, shows a mice with phenotype behavior of the Stage I (asymptomatic), similar to the behavior of the control’s mice, despite of have parasitaemia of 3%. The images 2d y 2e, represent to 80% of the mice with behavioral altered such as: without locomotor activity (score 0), flattened tail (score 0), pelvic elevation markedly fainted (score 0), piloerection (P), exhaustion reaction to finger pressure (score 0), with deviation to head (P), tremor (t), indicating that the...
mice were Stage III, moreover did they presented Blood Brain Barrier injured (Figure 3.3b). However, between the group of C57BL/6 mice, there was a 20% of the mice, that presented other scores to parameters such as: a exhaust reaction to finger pressure (score 1), deviation to head (A), tremor (2), and they didn’t presented injured brain barrier (Figure 3.3a), despite off to have parasitaemia of 28%, did not have neurological signs. Reports similar were presented by Martins et al., and Martínez et al.\(^8\) \(^11\)

In the photos 2f to 2i, can be observed the evolution of the phenotype behavior from the Stage I to Stage IV, in the group of the CB6F1 mice. This mice presented signs of the Cerebral Malaria as: in the straightening reflex, remains on the back (score 0), this behavioral not was presented by the other mice, in this condition wasn’t verified to observe injured of the brain blood barrier (Figure 3.4b), because the mice died with neurological alterations. All the mice CB6F1 presented the Stage IV.

In the Table 2, it is present a summary of the scores of parameters presented by the mice groups when they presented the parasitaemia percentage higher.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BALB/c</th>
<th>C57BL/6</th>
<th>CB6F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>locomotor activity</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>straightening reflex*</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>pelvic elevation</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>elevated tail</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>exhaust reaction to finger pressure</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>tremor</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>piloerection</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>deviation of head</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Parasitaemia (%)</td>
<td>60±4.6%</td>
<td>28±2.1%</td>
<td>24±3.2%</td>
</tr>
<tr>
<td>Mice with signs of MC</td>
<td>0/10</td>
<td>8/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Mice with brain blood barrier injury</td>
<td>0/10</td>
<td>8/10</td>
<td>10/10</td>
</tr>
</tbody>
</table>

* died mouse

The Figure 3, presented the mice brains of the three groups used in the assay, after of the perfusion with 2% of Evan’s Blue, can observed that the images 3b and 4b, presented a blue color, indicated Blood Brain Barrier injured.\(^11\)

In the Table 3, is presented a summary of the ranges of parasitaemia and days correlated with Cerebral Malaria Stage. How present the Table 3, the mice C57BL/6 y CB6F1, they started with signs Cerebral Malaria in the range of 6 to 7 days, with parasitaemias of 25% y 22% respectively, then the mice presented signs as: deviation of head and exhaust reaction to finger pressure with score between 1 to 0, and tremor con score de present (P). In these can observe too, that the mice BALB/c, presented the higher parasitaemia (60%), but not presented neurological signs of cerebral malaria, the correlation with the injured of blood brain barrier, determined the pathology. The results suggest that the infection with \textit{Plasmodium berghei} affect many neurological functions.\(^19\)

<table>
<thead>
<tr>
<th>Neurological stages</th>
<th>Mice groups</th>
<th>Days ranges</th>
<th>Ranges of percentages of parasitaemia</th>
<th>Number of mice affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>C57BL/6</td>
<td>0-3</td>
<td>0 - 11</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>CB6</td>
<td>0-4</td>
<td>0 - 10</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>0-6</td>
<td>0 –30 Major de 12%</td>
<td>10/10</td>
</tr>
<tr>
<td>Stage II</td>
<td>C57BL/6</td>
<td>3-5</td>
<td>11-20</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>CB6</td>
<td>4-8</td>
<td>10-23</td>
<td>8/10</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>6-11</td>
<td>Started en 30%</td>
<td>10/10</td>
</tr>
<tr>
<td>Stage III</td>
<td>C57BL/6</td>
<td>6-7</td>
<td>25</td>
<td>8/8*</td>
</tr>
<tr>
<td></td>
<td>CB6</td>
<td>8-9</td>
<td>22</td>
<td>10/10</td>
</tr>
<tr>
<td>Stage IV</td>
<td>CB6</td>
<td>9</td>
<td>24</td>
<td>3/5*</td>
</tr>
</tbody>
</table>

\(^*\) died mouse

Figure 3 Photos of the mice brains of the three groups used in the assay, treaties with evan’s blue solution. 1a. Brain of healthy mice. 2a y 2b. Brains of BALB/c mice parasitized. 3a y 3b. Brains of C57BL/6 mice parasitized. 4a y 4b. Brains of CB6F1 mice parasitized.
Conclusion

These results allow conclude that the SHIRPA protocols parameter used can determine functional and neurological progressive deterioration of parasitized mice. The C57BL/6 y CB6F1, both presented progressive demonstrations of cerebral malaria determined by behavioral alteration, started with locomotor activity diminished and terminate with head desviation and straightening reflex related with brain blood barrier injury. The CB6F1 group mice, presented severe manifestations of Cerebral Malaria characterized by lower parasitaemia than the group of C57BL/6 mice. The test carried out allowed to establish the patterns of functional and neurological behaviors with the percentages of parasitaemia, allowing to have a model that manifests cerebral malaria with low percentages of parasitaemia, as occurs in humans. At the same time it allowed to observe the differences in the behavior of susceptible and non-susceptible strains to the BALB/c, C57BL/6 mice and the CB6F1 mice that are produced in the INDICASAT AIP Bioterio.

Acknowledgements

The authors would like to thank everybody at the Center of the Cellular and Molecular Biology of Diseases - INDICASAT AIP, for providing the facilities and means to carry out this study Funds for this study were provided by SENACYT grants SNI 170-2016 and FID17-095.

Conflict of interest

Author declares that there is no conflict of interest in this study.

References


Citation: Jesus RD, Spadafora C. Evaluation of the malaria development in a group of CB6F1 mice. MOJ Bioequiv Availab. 2018;5(5):264–268. DOI: 10.15406/mojbb.2018.05.00112