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Research article

Plasmodium berghei MALARIAL INFECTION REDUCES BLOOD AND BRAIN GLUCOSE LEVELS IN EXPERIMENTAL MICE.

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Abstract

Glucose is a ubiquitous fuel and human's key source of energy, hence worthy of investigation during malarial infection. In this study, the changes in both blood and brain glucose levels induced by *Plasmodium berghei* malarial parasite in mice were investigated. Twelve male albino mice with an average weight of 26g were purchased and grouped into two(n=6). Group 1 mice were the control mice. The Group 2 mice were the experimental; infected with *Plasmodium berghei*. Both groups were fed with growers' mash and water *ad libitum*. On the sixth day, the animals were sacrificed under anaesthesia. Blood and brain samples were collectected and processed for biochemical assay using established standard procedure. Glucose levels were determined in both samples by documented method. Results show that serum glucose was significantly higher (P<0.05) in the control group (87.20±12.89mg/dl), compared with the experimental group (infected mice: 39.80±17.14mg/dl). Brain glucose level was also significantly higher (P<0.05) in Group 1 (control: 89.00±10.60mg/dl) compared with the Group 2 (infected mice: 41.80±4.12mg/dl).*Plasmodium berghei* infection reduces both blood and brain glucose levels in experimental mice. This observation may likely predict possible effect amongst humans. Therefore, human experience needs to be documented considering the important roles of glucose in brain function.

Keywords: malarial infection, mice, blood,

Introduction

Malaria is an enormous public health problem world wide and kills one to two million people every year in mostly sub-Saharan Africa (1). Further more, malaria is the most lethal parasitic disease in the world. Annually, it affects approximately 500 million people mostly in Africa's sub-Saharan countries (2).

Malaria infection in humans and animals is caused by the parasite *Plasmodium*. Several species of *Plasmodium* have the ability to cause malaria in animals, including rodents (mice). These parasites are not direct practical concern to man or his domestic animals. The interest of these parasites is that they are practical model organism in the laboratory for the experimental study of human malaria.

Plasmodium berghei is considered a comparable genetic model to human: There is a high degree of genetic conservation, this up to 99% (3) and it is well established that mice also exhibit natural differences in susceptibility to malaria infection (4). *P. berghei* is transmitted by *Anopheles* mosquitoes (transmits malaria also in humans) and it infects the liver after being injected into the blood stream by a bite of infected female mosquito. After a few days of development and multiplication, these parasites leave the liver and invade erythrocyte, (red blood cells).The multiplication causes anaemia and damage essential organs in the body. *P. berghei* infection also affects the brain and can cause cerebral complications in laboratory mice.

Glucose ($C_6H_{12}O_6$) is a simple monosaccharide found in plants. It is one of the three dietary monosaccharides, along with fructose and galactose, that are absorbed directly into the blood stream during digestion. Cells use it as energy and metabolic intermediate. Glucose is a ubiquitous fuel in biology. It is used as an energy source in most organisms from bacteria to humans. It is humans' key source of energy through aerobic respiration, providing approximately 3.75 kilocalories of food energy per gram (5). Through glycolysis and later in the reaction of the citric acid cycle (TCAC), glucose is oxidized to eventually form CO_2 and water, yielding energy sources, mostly in the form of ATP. Glucose is a primary source of energy in the brain.

Glucose levels are usually lowest in the morning, before meal, and rise after meals for an hour or two by few milligrammes. It is known that the brain glucose levels are 15-20% of blood levels (6, 7).

Blood glucose levels outside the normal range may be an indicator of a medical condition. A persistently high level is referred to as hyperglycaemia. Lower levels are referred to as hypoglycaemia (8). Hypoglycaemia can produce a variety of symptoms and effects but the principal problem arises from the inadequate supply of glucose to the brain resulting in impaired function (neuraglycopenia). Effect can range from mild dysphoria to more serious issue such as seizures, unconsciousness, and rarely permanent brain damage or death. Hypoglycaemia is often associated with malaria (9, 10, 11, 12). Human studies however concentrate on blood glucose.

This study evaluates changes in the levels of blood and brain glucose in *Plasmodium berghei* infected mice with the intention of correlating changes in blood with that of the brain and using observation in mice to predict possible human effect.

Materials and Method

Animal Care and Handling

Twelve (12) mice were purchased and maintained in the Laboratory Animal Centre of the Delta State University, Abraka, Nigeria. They were fed with commercial grower's mash and potable water for the entire duration of the study after acclimatizing for 7days.

Animal Grouping and Inoculation with *P. berghei*

A total of 12 albino mice weighing an average of 26 grams were used for the study. The mice were divided randomly into 2 groups (1 and 2) with 6 mice each in a group as follows: Group 1: Normal control (uninfected), Group 2: Parasitized (infected).

The animals were housed in plastic cages in a controlled condition of 12 hour light and 12 hour dark circle.

P. berghei infected mice from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos were used to infect the mice in Group 2. Drops of blood (prasitized) were mixed with normal saline in a ratio of 3:1.

From the mixture, 0.1 ml containing about 12,000 parasites was collected and injected intraperitoneally (IP) into the mice in Group 2, and kept for the newly inoculated mice to attained parasitaemia after 72hours and thereafter the experiment commenced.

Animal Sacrifice and Specimen Collection

The animals were sacrificed on the sixth day of the study after an overnight fast. The mice were anaesthetized with chloroform and blood samples were collected using a hypodermic needle and syringe from the ocular orbit into plain containers, then centrifuged for 10 minutes at 2,000 rpm to obtain serum.

The brain was isolated and homogenized in 5ml of freshly prepared normal saline. The mixture was centrifuged for 20 minutes at 2,000 rpm. The supernatant obtained was used for the experiment.

Analysis of Specimens

Glucose levels in both serum and brain specimens were determined using the glucose oxidase method as described by Hugget and Nixon (13). The commercial GLUC-PAP Reagent kit (Cat Number GL364) was supplied by Randox Laboratories, UK.

Results and Discussion

Results

The results obtained from the investigation into the effect of *Plasmodium berghei* malarial infection on the levels of both brain and blood glucose are presented in Table 1. Table 1 shows the Means ± SD of independent determinations of glucose in blood and brain, samples.

Table 1: Levels of brain and blood glucose induced by *Plasmodium berghei* malarial infection

Group	Serum Glucose (mg/dl)	Brain Glucose (mg/dl)
1	87.2 ± 12.89^{a}	89.0 ± 10.6^{a}
2	39.8 ± 17.14^{b}	41.8 ± 4.12^{b}

Values are expressed as Mean ± SD for n = 6 mice per group

Values that bear another superscript in a column differ significantly (P<0.05)

1 = Uninfected mice (control)

2 = Infected mice (infected with P. berghei malarial parasite)

The results(Table 1) showed significant decreases (P<0.05) in the levels of blood and brain glucose in malaria infected mice when compared with the control.

Discussion

This study concentrates on the levels of blood and brain glucose in experimental mice, inoculated with *Plasmodium berghei* malarial parasite. Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans of the genus *Plasmodium.*

The results obtained from this study reflects an interesting effect of malarial infection on blood and brain glucose levels in infected mice. Serum glucose was significantly higher (P<0.05) in Group 1 (control: 87.20±12.89mg/dl), compared with the Group 2 (infected mice: 39.80±17.14mg/dl). Brain glucose level was also significantly higher (P<0.05) in Group 1 (control: 89.00±10.60mg/dl) compared with Group 2 (infected mice: 41.80±4.12mg/dl).Both results showed significant decrease in the levels of blood and brain glucose in malaria infected mice when compared with the control.

Earlier workers reported the effect of malarial parasite infection on blood glucose in mice and humans. A research work carried out by Olayemi, *et al.* (12) on the "effect of malarial treatment on some biochemical parameter and plasma pH of mice infected with *Plasmodium berghei*", shows that blood glucose level ($mgdl^{-1}$) was

significantly lower (P<0.05) in the parasitized untreated mice (22.57±0.3) group compared with the control (33.74±0.1) group. This is in line with this present findings.

Severe malaria, particularly in children is often associated with hypoglycaemia (9) and blood glucose concentration can drop below 3mM (normal range 3.3 – 6.7mM). Young children living in sub-Saharan Africa carry the largest part of malaria burden (14) and hypoglycaemia is present in 20% of children and adults due to severe malaria (15). Hypoglycaemia is a cause of fatality in children and adult due to severe and complicated malaria.

White *et al*. (16) found out that 8% of their patients with cerebral malaria had reduced glucose levels (hypoglycaemia). Hypoglycaemia contributes significantly to the high mortality of cerebral malaria which ranges from 20 to 50% (17).

These observed hypoglycaemia could be due to the fact that *Plasmodium* parasites fully depends on glucose as a source of energy (18). Malaria parasites were discovered to be heterotrophic and dependent on glucose as a nutrient source, 100years ago (19). Glucose is rapidly taken up across the parasite plasma membrane through a facilitated hexose transporter and in turn metabolized through the process of glycolysis (20). This accompanied with approximately 100-fold increase in glucose utilization when compared with uninfected erythrocytes thus causing a profound hypoglycaemia when untreated (11).

Conclusion

This study reveals significant reductions in blood and brain glucose levels in *Plasmodium berghei* infected mice when compared with the control. The induced hypoglycaemia translates to the brain. This condition may adversely affect brain function since the brain largely depends on glucose as sole metabolic fuel. There is therefore the possibility of reduced brain glucose in man during malarial infection. This needs investigation, so that health care providers could be guided.

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