ALTERATION IN PLASMA AND HEPATIC GLUCOSE LEVELS INDUCED BY *PLASMODIUM BERGHEI* INFECTION IN EXPERIMENTAL MICE

Mordi J. C.

Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. E-mail: drjoechuks@gmail.com.

Accepted 21st May, 2020

Malaria poses a grave health problem in both tropical and sub-Saharan Africa. Postulations have connected this disease with hypoglycaemia which adds to its morbidity and mortality. Chloroquine is an anti-malaria drug largely applied in sub-Saharan Africa. In this study, the combined consequence of *Plasmodium berghei* malaria infection and chloroquine treatment on blood and hepatic glucose levels in mice was investigated to ascertain if both act synergistically to cause hypoglycaemia during malaria chemotherapy. Twenty-one albino mice of four months old and of average weight of 19g (13-25 g) were randomized into three groups (A, B and C) of seven mice each. Group A animals (control) received distilled water and feed only while mice in Group B were infected with *P. berghei* but were not treated. Group C animals were infected with the parasite and were treated with chloroquine (5 mg/kg body weight) for 5 days. Animals were sacrificed on the 6th day of the study after an overnight fast. Blood and hepatic glucose levels were assayed by the glucose oxidase method. Results show that *P. berghei* infection in mice reduced glucose levels in both blood (7.95 ± 0.61 mmol/L; P>0.05) and hepatic tissues (3.12 ± 0.37 mmol/L; p< 0.05) when compared with values (9.46 ± 1.32 mmol/L; 5.04 ± 1.36 mmol/ L, respectively) obtained from the uninfected mice. *P. berghei* infected mice treated with chloroquine had blood and hepatic glucose levels of 5.86 ± 1.08 mmol/L and 4.59 ± 0.6l mmol/L, respectively. Evidence indicates that chloroquine treatment of *P. berghei* malaria infection in mice further reduced the amounts of glucose when compared with the values obtained from the infected group that received no treatment. If animal to man extrapolation is permissible, then chloroquine chemotherapy could induce hypoglycaemia.

Key words: Hepatic glucose, malaria, *Plasmodium berghei*, plasma.

INTRODUCTION

Malaria, being a mosquito-borne infectious disease of humans and other animals, is caused by eukaryotic protist of the Plasmodium genus. In 2018, World Health Organization (WHO) estimated over 228 million cases of malaria with children under age five being the most vulnerable. Postulations from previous studies have associated malaria with reductions in glucose concentrations (Geoffrion et al., 1985; Sharma et al., 1992); however, hypoglycaemia is a common symptom in malaria as parasitized red blood cells utilize glucose 75 times faster than uninfected cells (MFl, 2000) and this is a tricky complication adding to the morbidity and mortality of malaria (Kakilaya, 2009).

Since malaria vaccines are not yet explored or remain problematic, chemotherapy continues to be the most potent weapon in the war against this epidemic disease (Turschner and Efferth, 2009), although supportive measures can help prevent transmission. Malaria is treated with drugs such as chloroquine, quinine, mefloquine, artesunate and others. The World Health Organization (WHO)'s recommendation of the intake of Artemisinin Combination Therapy (ACT) has been hampered by the imbalance between demand and supply in the poor socio-economically challenged rural population of sub-Saharan Africa, the epicenter of malaria infection. Chloroquine therefore continues to be used in most malaria ecological zones of developing countries despite the development of

Plasmodium falciparum resistance to the drug (WHO, 2006).

Several anti-malarial drugs have been demonstrated to affect glucose metabolism (La Fleur et al., 2001). It has been observed that quinine and quinidine cause hypoglycaemia during malaria therapy and mefloquinine even when used as a prophylactic, produced a similar effect (Davis, 1997). Some researches done on chloroquine to this effect have shown that chloroquine decreases blood glucose levels in animal models and patients without malaria infection (Powrie et al., 1991; Abdel-Gayoum et al., 1992; Gaafar et al., 2002). However, much literature was not found on the effect of chloroquine on glucose metabolism during malaria therapy. Studies reported that chloroquine lowered blood glucose levels and elevated hepatic glycogen in *Plasmodium berghei* infected mice (Murambiwa, 2011). Others indicated that chloroquine can spike up cellular uptake of glucose; however, the mechanism underlying remains equivocal (Zhou et al., 2016).

In this study, the consequence of chloroquine on blood and hepatic glucose concentration was investigated to determine if both factors act synergistically in malaria infection to produce hypoglycaemia, which can increase mortality in malaria infection. Blood and hepatic glucose levels were investigated because an understanding of the metabolic cascades of multicellular organisms at global level requires the study of the correlation among metabolic profiles of tissues and biofluid.

MATERIALS AND METHODS

A total of 21 animals weighing 13-25 g were used for the study. These were divided into 3 groups of seven per group. Group A animals were not infected with *P. berqhei* malaria parasite. Group B animals were infected with the parasite but were not treated, while Group C animals were infected with the parasite and were treated with standard chloroquine dose of 5 mg/kg/day as adopted by Izunya et al. (2011).

Inoculation Of Animals

Parasitic inoculation was adopted from previous studies (Abosi and Raseroke, 2003; Ogbonna et al., 2008). Three to four drops of parasitized blood were obtained from the tail of an infected blood by cutting the tip and then diluted with 0.9 ml phosphate buffer of pH 7.4. Furthermore, inoculation of test group mice was conducted intraperitoneally with 100 µl or 0.1 ml parasitized suspension. Parasitaemia was confirmed by making a thin blood film of blood collected from the cut-tip of the tail and then stained with Geimsa stain. Inoculation was performed in the Biochemistry Laboratory of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos.

Experimental design

After the confirmation of parasitaemia, the mice infected (parasitized) and none infected (normal) were divided into three groups of 7 mice per group and treated as follows:

Group A: Normal control
Group B: Parasitized control (*P. berqhei* treatment only)
Group C: Parasitized mice + chloroquine (5 mg/kg Body weight)

The administration of the chloroquine was carried out using an automated micropipette (oral cannula) for a period of five days. On the sixth day, mice were fasted overnight, and sacrificed. Their blood was collected for various biochemical estimations.

Preparation of plasma and tissues homogenate

Each mouse was anaesthetized in chloroform saturated chamber, and under such condition, excision of the thoracic and abdominal regions was conducted to reveal the heart and liver. Blood was obtained via heart puncture by means of a 5 ml disposable hypodermic syringe and needle and placed in ice-cold heparinized bottles. The liver was excised and a 20% homogenate in saline was prepared. The heparinized blood samples, liver homogenate was centrifuged at 1000 × g (Uniscope Model SM 902B Bench Centrifuge) for 10 min at room temperature in order to obtain plasma samples and tissue homogenates. They were collected and stored frozen until required for assay which was performed within 24 h.

Ethical consideration

As document and stipulated by Ward and Elsea (1997), ethical code of conduct with respect to animal care and regulation was adopted. The code of conduct was scrutinized, monitored and applied under strict observation. Likewise, the experimental procedures as depicted in this study were endorsed by an ad hoc ethical committee in the Faculty of Basic Medical Sciences, Delsu before the laboratory animals were used.

Biochemical assays

Plasma glucose concentration was estimated by adopting the glucose oxidase method (Barham and Trinder, 1972). All reagents employed for this assay were already reconstituted and packaged in commercial kits. Glucose kit was supplied by Randox Laboratories, United Kingdom.

Statistical analysis

The data obtained were expressed as Mean ± SD of seven mice per group. Differences between control and *P. berghei* treatment groups were compared using ANOVA and least square difference test (Lapin, 1978). P value <0.05 was considered significant.

RESULTS AND DISCUSSION

The results obtained from the investigation into the changes in blood and hepatic glucose levels in chloroquine sensitive *P. berghei* mice are shown in Table 1. Results indicated a significant reduction ($p < 0.05$) in hepatic glucose of the malaria infected mice (Group B). In the chloroquine treated group, there was a significant increase in hepatic glucose level when compared with the malaria infected mice alone; however, this increase was not statistically significant ($p>0.05$) when compared with the control group. There was also a decrease in the blood glucose levels of the infected with *P. berghei* when compared with the control, but this was not statistically significant ($p>0.05$). In the chloroquine treated mice, blood glucose levels revealed a decrease which when compared with the control group and the malaria infected mice alone (Group B), was statistically significant ($p<0.05$).

The result of this study showed significant reduction (61.5%) in hepatic glucose in the malaria infected mice when compared with the normal mice. Similar results were observed by Geoffrion et al. (1985) and Sharma et al. (1992). A related study evaluated the liver gluconeogenic activity of *P. berghei* infected mice and observed that the glucose in the liver of the infected mice was significantly reduced (56%) when compared with the glucose in the livers of normal animals (Geoffrion et al., 1985). Furthermore, previous studies have reported a significant depletion of carbohydrates, glycogen and glucose content in the liver at high parasitaemia (Sharma et al., 1992). Other studies by MFI (2000) and Jia et al. (2008) also reported such depletions of glucose in malaria infection.

Table 1. Hepatic and blood glucose levels in normal, *P. berghei* infected and chloroquine treated mice.

Groups/Treatment	Hepatic glucose (mmol/L/g wet tissue)	Blood (Plasma) Glucose (mmol/L)
A (normal)	5.04± 1.36	9.46± 1.32
B (infected not treated)	3.12±0.37*	7.95±0.61
C (infected and treated)	4.59±0.61	5.86 ±1.08*

n= 7; values are expressed as Mean ± SD. * Depicts values significantly different from the control (group A) at p<0.05.

The results obtained from this study tend to corroborate previous studies by depicting a significant decrease ($p<0.05$) in hepatic glucose upon treatment with chloroquine when compared with the infected mice without treatment. Chloroquine administration nevertheless could not restore hepatic glucose to normal value in control mice. Gaafar et al. (2002), in a study to determine the effect of chloroquine on glucose metabolism, reported an increase in glycogen levels. The result of this research is similar to that of Gaafar et al. (2002) and Murambiwa (2011) findings as an increase in hepatic glucose could

lead to its deposition in glycogen stores, thereby elevating hepatic glycogen levels.

In plasma (blood), there was a marked depletion of glucose ($p < 0.05$) in the chloroquine treated malaria infected mice when statistically contrasted with the control and untreated group (Table 1). The result observed from the aforementioned result tends to correspond with studies conducted by Powrie et al. (1991), Abdet - Gayoun et al. (1992), Gaafar et al. (2002) and Murambiwa (2011). Postulations from these studies, however, estimated that chloroquine administration to *P. berghei* infected rats resulted in the lowering of blood glucose levels. One explanation for the decrease in blood and hepatic glucose levels is that parasitized red blood cells utilize glucose ($C_6H_{12}O_6$) more than the uninfected cells. This is because at the intraerythrocytic asexual stage, the parasite lacks a functional tricarboxylic acid (TCA) cycle. It therefore depends on the host red blood cell for energy supply, obtained primarily through anaerobic glycolysis (Lang-Unnasch and Murphy, 1998; Mehta et al., 2005). This increase in glucose levels is about 75 times higher than that in normal cells (MFI, 1999). The reduction in blood glucose level may have been caused by the breakdown of the glycogen stores in the liver and depletion in the liver glucose in a bid to restore blood glucose homeostatic levels, as the liver acts as a glucose buffer.

When blood glucose level falls below normal, processes like glycogenolysis, gluconeogenesis, lipolysis and others are metabolically mobilized to normalize blood glucose homeostasis. This actually may be the reason for the insignificant decrease in blood glucose. Hence, the decrease in hepatic glucose was however significant because the liver in an attempt to return blood glucose to normoglycaemic levels may make its glucose available in the blood, so it would be available for extrahepatic cells.

Consequently, the significant decrease in blood glucose levels with simultaneous reduction of hepatic glucose levels upon the administration of chloroquine might be due to the ability of chloroquine to increase insulin activity. Studies by Gaafar et al. (2002) have hypothesized the insulin increasing activity of chloroquine which has further been corroborated by other reports that chloroquine increases C-peptide secretion (a by-product of insulin production) (Powrie et al., 1991; Pestana et al., 2015).

Increase in insulin secretion causes the mobilization of blood glucose to hepatic and extrahepatic cells. In the hepatic cells, the excess glucose may then be used either for glycogen synthesis or converted to acetyl-CoA which is used for fatty acid synthesis (Halaby et al., 2013). This mobilization of glucose from the blood may be the cause of the decrease in blood glucose and increase in hepatic glucose levels. Chloroquine may therefore further increase the hypoglycaemia caused by malaria by inducing the secretion of insulin (Halaby et al., 2013). In addition, studies by White et al. (1983) confirm that drug of quinine origin has the potentials of precipitating hypoglycaemia in malaria infection through this mechanism. These findings indicate that chloroquine administration in malaria therapy may work synergistically with malaria infection to further increase hypoglycaemia which according to kakilaya (2009) is a major cause of death in malaria infection. Although there are associated problems with chloroquine usage in malaria treatment (because of increase resistance), these findings are still relevant and applicable as chloroquine chemotherapy is one major drug currently adopted in the treatment of malaria in poor socio economically challenged rural populations of sub-Saharan Africa, the epicenter of malaria infection.

Further investigation should therefore be undertaken to determine the rate of death among malaria patients treated with chloroquine so as to ascertain if there is a significant relationship between hypoglycaemia induced by chloroquine when used in malaria treatment and the number of death cases in malaria infection.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

ACKNOWLEDGEMENTS

The author wishes to thank the Nigeria Institute

of Medical Research, Yaba, Lagos for providing the stock of *Plasmodium berqhei* and also grateful to the staff of African Research Laboratory, Isiokolo for their assistance during the assay period.

REFERENCES

Abdel-Gayoum, A. A., Haider, S. S., Tash, F. M and Ghawarshe, A. (1992). Effect of chloroquine on some carbohydrate metabolic pathways: content of GSH, ascorbate, and lipid peroxidation in rats. *Pharmacology and Toxicology* 71: 161-164.

Abosi, A. O., and Raseroke, B. H. (2003). *In vivo* anti-malarial activity of *Vernonia amygdalina*. *British Journal of Biomedical Science* 60: 89-91.

Davis, T. M. E. (1997). Anti-malarial drug and glucose metabolism. *British Journal of Clinical Biochemistry* 44: 1-7.

Gaafar, K. M., Khedr, M. I., Bashandy, A. S., Sharaf, A. O. and el-Aayat, R. S. (2002). Effect of chloroquine on glucose metabolism. *Arzneimittelforschung* 52: 400 - 406.

Geoffrion, V., Butter, K., Pass, M., Smith, C. I. and R. Deslauriers. (1985). *Piasmodium berghei*: gluconeogenesis in the infected mouse liver studied by nuclear magnetic resonance. *Experimental parasitology* 59: 364-374.

Halaby, M.J., Kastein, B.K. and Yang, D.Q. (2013). Chloroquine stimulates glucose uptake and glycogen synthase in muscle cells through activation of AKT. *Biochemistry and Biophysiological Research Communication* 435:708 - 713.

Izunya, A. M., Nwaopara, A.O., Anyanwu, L. C., Odike, M.A.C., Oaikhena, G. A., Bankole, J.K. and Okhiai, O. (2011). Effect of chronic oral administration of chloroquine on the histology of the heart in Wistar rats, *Biology and Medicine*, 3 (4): 01-06.

Jia, V. L., Yulan, W., Jasmana, S., Jeremy, K. N., Steohen, D., Burton, H. S., Marcel, J., Sergio, W., Elaine, H. and Jurg, U. (2008). Global metabolic response of NMRI mice to an experimental *Plasmodium berghei* infection. *Journal of Proteome Research* **7**: 3945-3956.

Kruckeberg, C.W., Sander, B. J. and Sulbuan, C. D. (1981). *Plasmodium berghei*: glycolytic enzymes of the infected mouse erythrocyte. *Experimental Parsitology* 51: 438 - 443.

La Fleur S. E., Kalsbeek, A. Wortel, J. Fekkes, M. L. and Buijs, M. R. (2001). A daily rhythm in glucose tolerance, a role for the suprachiasmatic nucleus. *Diabetes* 50: 1237-1243.

Lang-Unnasch, N., and Murphy, A. D. (1998). Metabolic changes of the malaria parasite during transition from the human to the mosquito host. *Annual Review of Microbiology* 52: 561-590.

Malaria Foundation International (MFI). (2000). Malaria Glossary. Retrieved May 17, 2018, from http://www.malaria.org/malariaglossary.html.

Mehta, M., Sonawat, H. M. and Sharma, S. (2005). Malaria parasite infected erythrocytes inhibit glucose utilization in uninfected red cells. *FEBS Letters* 578: 6151- 6158.

Murambiwa, P. (2011). Effect of novel chloroquine formulation on blood glucose concentration, renal and cardiovascular function in experimental animal paradigms. Ph.D. dissertation, University of KwaZulu-Natal, Westville, South Africa.

Ogbonna, D. N., Sokari, T. G. and Agomuoh. A. A. (2008). Anti-malarial activities of some selected traditional herbs from South Eastern Nigeria against *Plasmodium* species. *Research Journal of Parasitology* 3: 25-31.

Pestana, C.R., Oishi, J. C., Salistre-Araujo, H. S. and Rodrigues, G. J. (2015). Inhibition of autophagy by chloroquine stimulates nitric oxide production and protects endothelial function during serum deprivation. *Cell Physiol Biochem* **37**:1168 - 1177.

Powrie, J. K., Smith, D. G., Shojaee-Morade, F., Sonksen, H. P. and Jones, H. R. (1991). Mode of action of chloroquine in patients with non-insulin-dependent diabetes mellitus. *America Journal of Physiology, Endocrinology and Metabolism* 260: E897-E904.

Sharma, N., Mandi, A. A., Khanna, R., Khan, H. M., Kumar, H. and Ahmed, S. (1992). Biochemical study of mouse liver following *Plasmodium berghei* infection. *India Journal of Medical Research* 95**:** 84-87.

Trinder, P. (1969). Determination of glucose in blood using glucose with alternative oxygen acceptor, *Annual Clinical Bioch*emistry 6: 24

Turschner, S. and Efferth, T. (2009). "Drug resistance in plasmodium: natural products in the fight against malaria," Mini-Reviews in Medicinal Chemistry, 9 (2): 206 –214.

Ward, J. W. and Elsea, J. R. (1997). Animal case and use in drug fate and metabolism. In: Edward RG, Jean LH (eds) Methods and techniques, 1st edn., New York: Markel Dekker. 114.

White, N. J., Warrell, D. J., Chantavanich, P. L., Looareesuwan, S., Warrell, M. I., Krishna, S., Williamson, D. and Turner, R. C. (1983). Severe hypoglycaemia and hyperinsulinaemia in *Plasmodium falciparum* malaria. *New England Journal of Medicine.* 309: 60-66.

WHO Report (2011). "World Malaria Report" Geneva. http://www.who.int/malaria/wmr

Zhou, Q., Yang, X., Xiong, M., Xu, X., Zhen, L., Chen, W., Wang, Y., Shen, J., Zhao, P. and Liu, Q. H. (2016). Chloroquine Increases Glucose Uptake via Enhancing GLUT4 Translocation and Fusion with the Plasma Membrane in L6 Cells. *Cellular Physiology and Biochemistry*; 38:2030 - 2040 https://doi.org/10.1159/000445562.