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The alteration of biochemical parameters leading to organ damage during *Plasmodium berghei* ANKA infection in miceSukanya Pattarapo¹, Kittikarn Ratanavijarn¹, Voravuth Somsak^{2*}¹Department of Clinical Chemistry, Faculty of Medical Technology, Western University, Kanchanaburi 71170, Thailand²Department of Medical Technology, School of Allied Health Science, Walailak University, Nakhon Si Thammarat 80161, Thailand

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ABSTRACT

Objective: To investigate the alteration of biochemical parameters during *Plasmodium berghei* ANKA (*P. berghei* ANKA) infection in mice.**Methods:** Male BALB/c mice were intraperitoneally inoculated with 1×10^7 parasitized erythrocytes of *P. berghei* ANKA. Parasitemia was daily monitored by microscopy of Giemsa stained thin blood smear. Additionally, packed cell volume (PCV) and biochemical parameters including glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, and bilirubin levels were also measured using Cobas 111 automate analyzer.**Results:** Parasitemia was increased during *P. berghei* ANKA infection until the infected mice died within 2 weeks, and early and late infections were separated by Days 4 and 10, respectively. At early infection, it was found that hemolysis occurred as indicated by markedly decreased PCV. Hypoglycemia and acute kidney injury were also developed at the early infection as indicated by decrease in blood glucose and increase in BUN and creatinine levels. Moreover, the loss of liver function was observed at the late infection as indicated by markedly increased enzyme activities of AST, ALT, and ALP, and decreased albumin level. Additionally, bilirubin level was also increased.**Conclusions:** The finding reveals the pathological condition during *P. berghei* ANKA infection in mice. The hemolysis and acute kidney injury were developed at the early infection, and loss of liver function then occurred at the late infection. Hence, the prevention of these pathological conditions during malaria infection is urgently needed.

1. Introduction

Malaria is a parasitic disease caused by protozoa parasite of genus *Plasmodium* and transmitted by the bite of female *Anopheles* mosquito. Malaria still remains a serious infectious disease in tropical and sub-tropical areas such as Africa, South and Central America, and Asia with estimates of 500 million cases annually, especially with children younger than 5 years of age

and pregnant women[1]. During malaria infection, the increase in production of reactive oxygen species has been observed in the experiments and patients with falciparum malaria[2,3]. Malaria-associated biochemical parameter alteration and organ dysfunction, one of the major life-threatening causes of death, occurs in 5%–10% of hospitalized patients[4]. The malaria-associated hypoglycemia, acute kidney injury, and loss of liver function followed by death are indicated by critical alteration of biochemical parameters including blood glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, and bilirubin, respectively. Therefore, this study aimed to investigate how *Plasmodium berghei* ANKA (*P. berghei* ANKA) infection affects or modifies biochemical markers leading to organ damage and death.

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All the experiments involving mice were conducted in accordance to the International Animal Ethics Committee NIH Guidelines and approved by the Animal Ethical Committee, Western University, Thailand (WTU-AE-4328).

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2. Materials and methods

2.1. Experimental mice

Pathogen-free male BALB/c mice aged 6–8 weeks old weighting 20–25 g used in this study were obtained from the National Laboratory Animal Center (NLAC), Mahidol University, Bangkok, Thailand. They were kept in the mouse room with temperature control between 22–25 °C, and 12 h light/12 h dark cycle. The standard pelleted diet (CP082, Perfect Companion Company, Bangkok, Thailand) and clean drinking water were provided *ad libitum*. All the experiments involving mice were conducted in accordance to the International Animal Ethics Committee NIH Guidelines and approved by the Animal Ethical Committee, Western University, Thailand (WTU-AE-4328).

2.2. Rodent malaria parasite

Chloroquine-sensitive strain of *P. berghei* ANKA used in this study was obtained from Malaria Research and Reference Reagent Resource Center. The frozen stock of the parasite was inoculated intraperitoneally to naïve BALB/c mice. Parasitemia was determined by microscopy of Giemsa stained thin blood smear. *P. berghei* ANKA was maintained by mechanical serial passage of 1×10^7 parasitized erythrocytes on a weekly basis. Percentage of parasitemia was calculated using the formula below:

$$\% \text{ Parasitemia} = \frac{\text{Number of parasitized erythrocytes}}{\text{Number of total erythrocytes}} \times 100$$

2.3. Measurement of packed cell volume (PCV)

PCV was measured by collecting tail blood of each mouse in heparinized capillary tubes. The tail blood was filled up to three fourths of the tube whose one end was sealed with crystal seal properly. Centrifugation was subsequently performed at 5000 r/min for 5 min. PCV was then calculated using the formula below:

$$\% \text{ PCV} = \frac{\text{Volume of packed erythrocytes}}{\text{Total blood volume}} \times 100$$

2.4. Measurement of biochemical parameters

Blood samples were collected by cardiac puncture into heparinized vacuum tubes. Centrifugation was done at 5000 r/min for 15 min, and plasma was then collected into new sterile test tube. The biochemical parameters including glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, and bilirubin were measured by Cobas C111 automate analyzer (Roach Diagnostics Limited, Switzerland).

2.5. Statistical analysis

The results were expressed as mean \pm SEM. Significance was considered at 95% confidence, $P < 0.05$ using One-way ANOVA with *post-hoc* Tukey test. All statistical analyses were done by GraphPad Prism software (GraphPad Prism Software, Inc., US).

3. Results

3.1. Propagation of *P. berghei* ANKA infection in mice

As showed in Figure 1A, parasitemia was increased from Day 1 post infection until Day 14 (65%). This marked increase was firstly observed on Day 4 with a parasitemia of 15%. While % parasitemia was increasing in mice, increase in hemolysis was observed as indicated by decrease in PCV (Figure 1B). Additionally, *P. berghei* ANKA infected mice died on Day 14 post infection (Figure 1C).

3.2. *P. berghei* ANKA-associated hypoglycemia and acute kidney injury development in mice

P. berghei ANKA infection significantly ($P < 0.05$) decreased levels of blood glucose on Day 4 post infection (Figure 2A). Moreover, it was observed that BUN levels were significantly ($P < 0.05$) increased on Day 6 post infection (Figure 2B), while increased levels of creatinine were observed and reached significances ($P < 0.05$) on Day 8 post infection (Figure 2C).

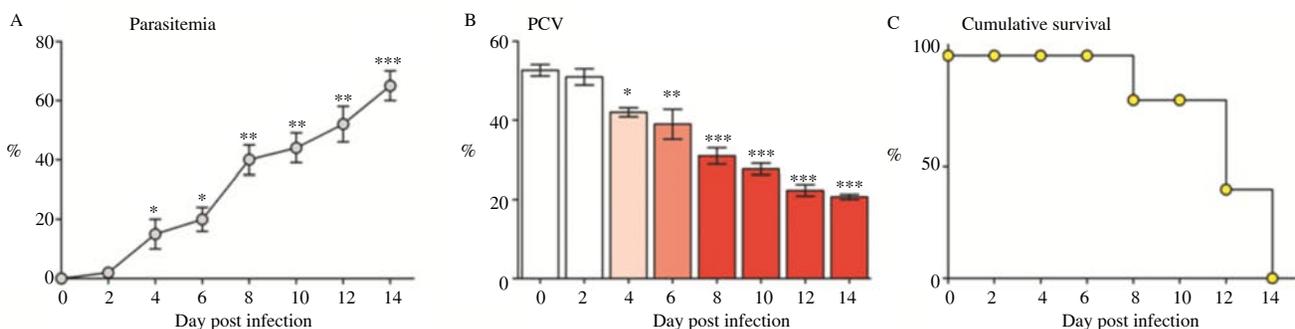


Figure 1. Propagation of *P. berghei* ANKA infection in mice.

A: Parasitemia; B: PCV levels; C: Cumulative survival. Results were expressed as mean \pm SEM. *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$ compared to Day 0 post infection.

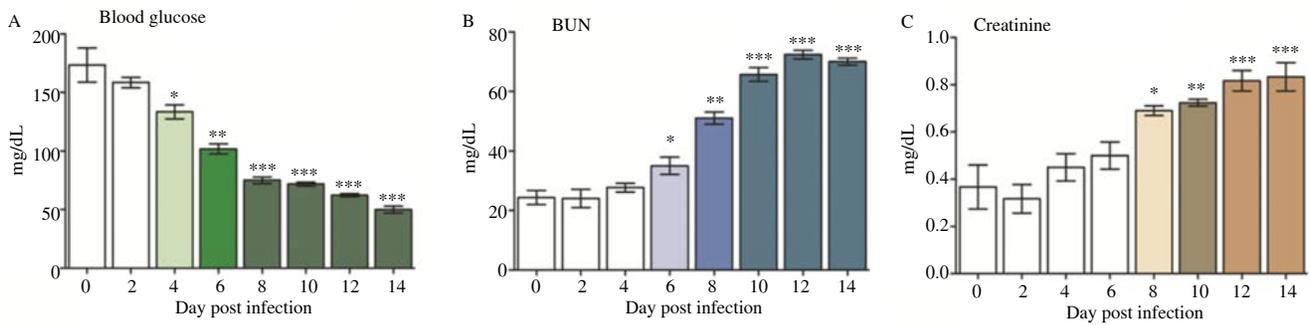


Figure 2. *P. berghei* ANKA-associated hypoglycemia and acute kidney injury in mice.

A: Blood glucose; B: BUN; C: Creatinine levels. Results were expressed as mean \pm SEM. *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$ compared to Day 0 post infection.

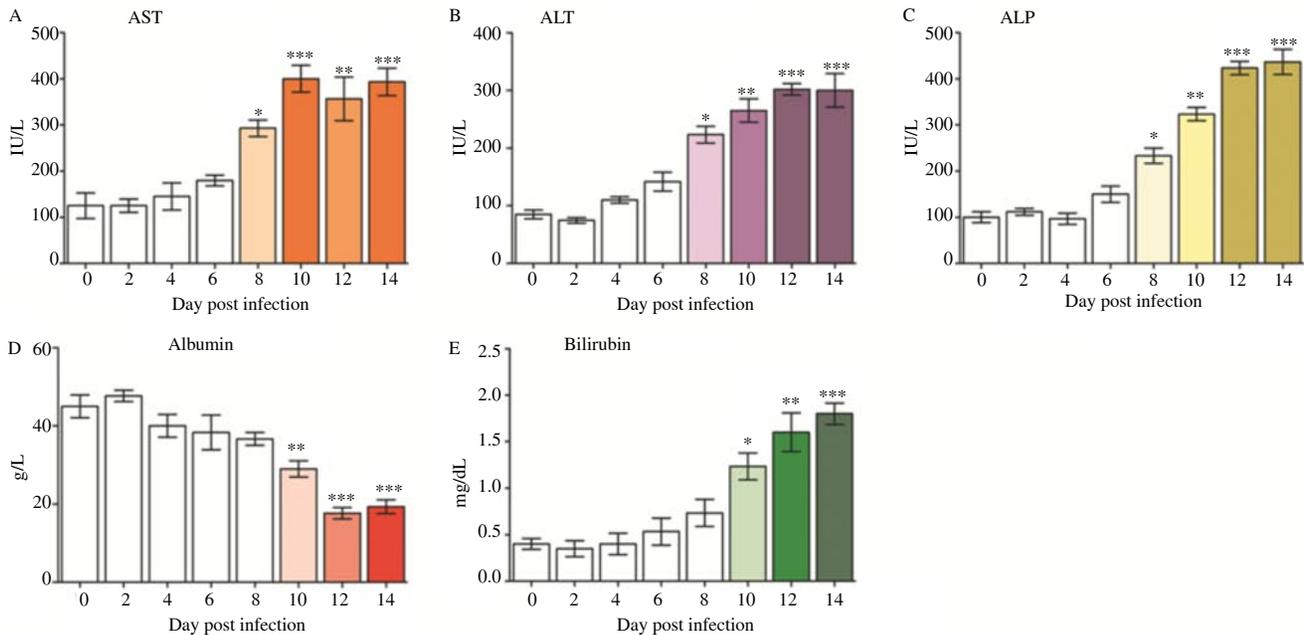


Figure 3. *P. berghei* ANKA-associated loss of liver function in mice.

A: AST; B: ALT; C: ALP; D: Albumin; E: Bilirubin levels. Results were expressed as mean \pm SEM. *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$ compared to Day 0 post infection.

3.3. *P. berghei* ANKA-associated loss of liver function in mice

Liver enzyme activities of AST, ALT, and ALP showed a progressive increase in response to the presence of parasite, which reached significance ($P < 0.05$) on Day 8 post infection (Figure 3A–C, respectively). Decreased albumin levels were observed with significant values ($P < 0.01$) on Day 10 post infection (Figure 3D). Additionally, it was observed that levels of bilirubin were significantly ($P < 0.05$) increased at Day 10 post infection (Figure 3E).

4. Discussion

The present study has provided evidence that describes alteration in the biochemical parameters related to pathology of hypoglycemia, acute kidney injury, and loss of liver function in *P. berghei* ANKA infection in mouse model. These pathological

conditions during malaria infection have been notified by clinical reports, and they have been important life-threatening complication of malaria infection[5]. The difficulties in access to medical services or delay in diagnosis are implicated in the severity of disease. While in blood stage propagation of *P. berghei* ANKA, PCV was markedly decreased due to hemolysis of erythrocytes and insufficient mature erythrocyte production[6]. In addition, oxidative stress has been found to develop in both parasitized and non-parasitized erythrocytes during *P. berghei* ANKA infection resulting in the decrease of survival time of the erythrocytes[7]. Furthermore, malaria infection has been found to correlate with the incidence of erythrocyte destruction[8].

The onset of hypoglycemia in *P. berghei* ANKA infected mice came out from Day 4 post infection as confirmed by decreased blood glucose. It could be due to the fact that during malaria infection in erythrocytic stage, glucose was rapidly taken up across the parasite membrane through a facilitated hexose transporter[9]. This was accompanied with an approximately 100-fold increase in

glucose utilization when compared with normal erythrocytes, thus causing hypoglycemia if untreated[10].

The incidence of acute kidney injury was confirmed by increased BUN and creatinine levels in *P. berghei* ANKA infected mice. Additionally, significant increases in liver enzyme activities such as AST, ALT, and ALP, and markedly decreased albumin levels as well as increased bilirubin levels were also observed during malaria infection. Malaria-associated organ damages including acute kidney injury and loss of liver function with the involvement of parasitized erythrocyte adherence, proinflammatory response and oxidative stress have been hypothesized[11-13]. The consumption of hemoglobin by malaria parasites and the erythrocyte destruction give rise to toxic free heme that has the ability to induce oxidative stress during *P. berghei* ANKA infection[14]. In addition, the oxidative stress has been implicated in lipoprotein oxidation and serious damage in different organs such as kidney and liver through the generation of reactive oxygen and nitrogen species[15]. Hence, these conditions derived by *P. berghei* ANKA infection result in acute kidney injury and loss of liver function[11,16,17].

The results obtained in the present study demonstrated that parasite propagation, hemolysis, hypoglycemia, acute kidney injury, and loss of live function during *P. berghei* ANKA infection in mice were observed. They are important life-threatening complications of malaria infection. Therefore, strategies for protection and treatment of these complications induced by malaria are urgently needed.

Conflict of interest statement

We declare that we have no conflict of interest.

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