

Effect of green tea extract on blood glucose levels during *Plasmodium berghei* infection in mice

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Abstract

Hypoglycemia is recognized as a serious complication of malaria during *Plasmodium* infection in blood stage. Hence, this study was aimed to investigate the effect of green tea extract on blood glucose levels during *Plasmodium berghei* infection. Groups of naïve ICR mice were inoculated with 1×10^6 parasitized erythrocytes of *P. berghei* ANKA. Green tea extract (3,000 mg/kg) was given orally twice a day for 6 consecutive days, and blood glucose levels were subsequently measured. The results showed that levels of blood glucose were decreased significantly during malaria infection on day 6. Interestingly, blood glucose was maintained into normal levels in green tea extract treated PbANKA infected mice. It can be concluded that green tea extract was exerted glucose homeostasis during malaria infection.

Keywords: blood glucose levels, green tea extract, *Plasmodium berghei*

1. Introduction

Malaria is responsible for 300-700 million cases worldwide annually, especially tropical and sub-tropical areas. It can be estimated that 90% of all malaria deaths occur in Africa, most commonly in children under the age of 5 (Mathanga et al., 2012). Malaria is caused by protozoa parasite in genus *Plasmodium*. Infection is initiated when *Plasmodium* Sporozoites are injected into bloodstream of a vertebrate host by an infected female *Anopheles* mosquito, and travel to the liver. During hepatocyte infection, differentiate and divide asexually to produce merozoites are occurred. Upon maturation, merozoites are released and invaded erythrocytes

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following initiate the blood stage of infection, which is responsible for the symptoms of the disease (Vaughan and Kappe, 2012). In most cases, malaria deaths are related to one or more of these serious complications such as cerebral malaria, severe anemia, breathing problem and organ failure (Tangpukdee et al., 2009). The pathology and consequences of infection in the individual complications are indeed variable, but a common finding in all complications is the metabolic disturbance, importantly of glucose. Glucose metabolism during malaria infection is in part influenced by the intra-erythrocytic malaria parasite, and glucose is a source of energy to support parasite growth and survival (Elased et al., 1996). It has been found that blood glucose transport across erythrocyte membrane was increased during blood stage infection resulting to hypoglycemia, and people with acute hypoglycemia might die from hypoglycemic shock (Eltahir et al., 2010). In this respect, plant resources are potential targets for research and development of alternative ways to solve this problem. Green tea (*Camellia sinensis*) is widely used as favorite beverage. Catechins, major active component in green tea extract, represent 15% of plant dry weight. They have been reported to exert several activities including antioxidant, anti-cancer, anti-inflammation and anti-microbial (Yang and Pan, 2012). Moreover, green tea extract has been reported the lowering effect on blood glucose level in mice (Wein et al., 2013). Hence, this study was aimed to investigate the effect of green tea extract on glucose levels during *P. berghei* infection in mice.

2. Materials and Methods

2.1 Green tea material

Fresh leaves of green tea (*Camellia sinensis*) were obtained at the Royal Project shop, Chiang Mai province, Thailand. The voucher specimen has been deposited in the Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. The plant was air-dried at room temperature and subsequently powdered. Dried leaves of green tea (10 g) were used to prepare crude extract with 100 mL of water using hot water method with constant temperature of 95°C for 15 Min (Vuong et al., 2011) and contained 60% of total polyphenols, >40% of EGCG, and <0.1% of caffeine by HPLC.

2.2 Experimental animal

For this study, female outbred ICR mice between 4-6 weeks of age were obtained from National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. They were kept in

a 12 h light/12 h dark cycle with 22-25°C and given standard mouse pelleted diet (CP diet 082, Perfect Companion Company, Bangkok, Thailand) and clean water *ad libitum*. Procedures of the animal experiments were ratified by the Ethical Committee on Animal Experimentation, Faculty of Medical Technology, Western University, Kanchanaburi, Thailand.

2.3 Rodent malaria parasite

Chloroquine-sensitive strain of *Plasmodium berghei* ANKA (PbANKA) was used. Frozen parasite from stock in liquid N₂ was warmed at 37°C and then inoculated by intraperitoneal (IP) injection into naïve ICR mice before experiments. Parasitemia was daily monitored by microscopy of Giemsa stained thin blood smear and calculated using formula below.

$$\% \text{Parasitemia} = \frac{\text{No. of infected erythrocytes}}{\text{No. of all erythrocytes}} \times 100$$

For mechanical sub-passage, infected blood with a parasitemia of 10-15% was collected by cardiac puncture and IP injection of 200 µL containing 1x10⁶ infected erythrocytes was subsequently performed into naïve mice using phosphate buffer saline (PBS) as solvent.

2.3 Hematocrit level measurement

Percent hematocrit (%Hct), also called packed-cell volume, was measured to determine levels of packed erythrocytes and anemia. Tail blood was collected in heparinized capillary tube and centrifugation was subsequently performed at 10,000 g for 10 Min. Finally, proportion of packed erythrocytes and total blood volume was calculated.

2.4 Antimalarial drug

Standard antimalarial drug used in this study was chloroquine (CQ). This drug was freshly prepared in distilled water (DW) and administered orally by gavage. Drug dose, expressed in mg/kg of body weight, was adjusted at the time of administration according to the weight of each mouse. The dose was based on sub-curative dose of this drug on PbANKA infected mice.

2.5 Blood glucose level measurement

One hundred μ L of tail blood was collected in heparinized capillary tube. Centrifugation was then performed at 10,000 g for 10 Min, and plasma was subsequently collected into a new 1.5-mL microcentrifuge tube. Level of glucose in plasma was measure using commercial kit (Biosystem S.A. Costa Brava, 30 Barcelona, Spain), according to a manufacturer's instruction. These markers were measured daily during infection.

2.6 Efficacy test of green tea extract *in vivo*

For *in vivo* test, the experiment was based on standard Peter's test (Peters, 1975). Groups of ICR mice (5 mice in each group) were inoculated with 1×10^6 parasitized erythrocytes of PbANKA by IP injection. Green tea extract (3,000 mg/kg body weight of the animal) was given orally by gavage twice a day for 6 consecutive days. Percent parasitemia, %Hct, and glucose level in plasma were measured as previously described. The control groups were included; the uninfected and infected controls were treated with DW, and drug treated control was given CQ (5 mg/kg).

2.7 Statistical analysis

The results were expressed as mean \pm standard error of mean (SEM). Statistical significance was assessed by one-way ANOVA or, when appropriate and Student's *t* test for paired observation. Values of $p < 0.05$ were considered significant.

3. Results and Discussion

3.1 Blood stage propagation of PbANKA infected mice

Having confirmed that PbANKA developed normally *in vivo*, we examined the blood stage phenotype of this parasite. The results showed that parasitemia was developed normally and first detectable on day 2 (<1%), and parasitemia was increased onward (Fig 1a). Moreover, during parasite growth, %Hct was decreased and negative correlation with % Parasitemia was also observed (Fig 1b-c). PbANKA has a preference for invading reticulocytes, however, at low parasitemia majority of this parasite can be found inside mature erythrocytes. At the levels of parasitemia increase from day 7, there is a marked increase in circulating reticulocytes in response to the decrease in erythrocytes levels caused by hemolysis during merozoites released into bloodstream (McNally et al., 1992). Therefore, increasing of parasitemia with decreasing of

hematocrit during blood stage propagation of PbANKA lead to animal die from hemolytic anemia on day 10 (Fig. 1).

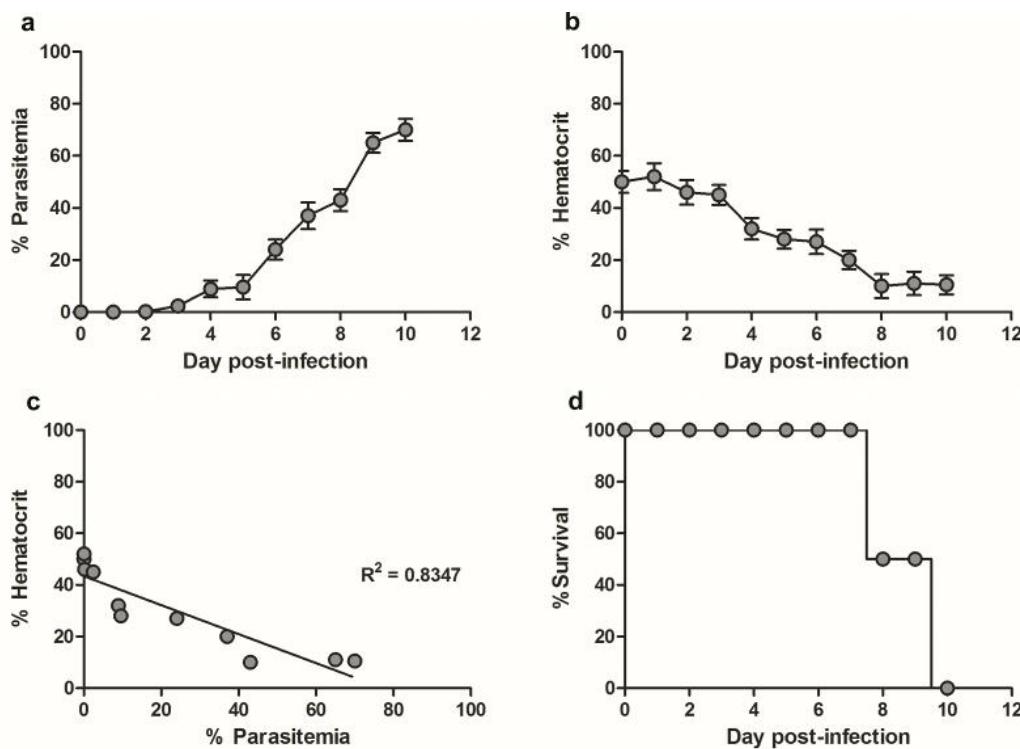


Figure 1. Propagation of blood stage *Plasmodium berghei* ANKA infection in ICR mice. Naïve ICR mice were inoculated with 1×10^6 parasitized erythrocytes of PbANKA, and (a) %parasitemia, (b) %hematocrit, (c) correlation of %parasitemia and %hematocrit, and (d) survival of infected mice were subsequently determined. Results were expressed as mean \pm SEM.

3.2 Blood glucose levels during PbANKA infection in mice

For determining of hypoglycemia during malaria infection, glucose level in plasma was measured (Fig. 2a). Blood glucose was decreased significantly ($p<0.05$) on day 6 after infection, and severe hypoglycemia occurred 1-2 days before death, when the parasitemia around 70%. Furthermore, strong negative correlation ($R^2=0.9290$) between parasitemia and glucose level in plasma was observed (Fig. 2b). In contrast, correlation of hematocrit and glucose level was observed in strong positive correlation ($R^2=0.9234$) (Fig. 1c). It can be suggested that during malaria infection in blood stage, glucose in erythrocytes was used to supply the energy and survival of parasites. So, increasing of glucose transport across erythrocyte membrane has been

described via glucose transporter (GLUT) resulting hypoglycemia in bloodstream (Joet et al., 2003). The exact mechanism of hypoglycemia in malaria remains controversial, but insulin appears to play a role in rodent models. It has been reported that hypoglycemia and hyperinsulinemia were only detected when parasitemia exceeded about 20%, which may explain why hypoglycemia was found on day 6, when parasitemia rose to about 25-30% (White et al., 1983; Das et al., 1988; Elased et al., 1996; Eltahir et al., 2010). In addition, high levels of tumor necrosis factor (TNF) were responsible for malaria infection, and increase glucose uptake by infected erythrocytes (Elased et al., 1996).

3.3 Efficacy of green tea extract on glucose levels during PbANKA infection

In order to solve the hypoglycemia problem during malaria infection, green tea extract was tested against PbANKA. As showed in Fig. 2d, green tea extract presented anti-hypoglycemia during PbANKA infection. Hypoglycemia was observed in untreated group with a significant ($p<0.01$), compared to normal group. However, normal glucose level was found during PbANKA infection and given green tea extract twice a day in GTE group. It can be suggested that catechins in green tea extract were exerted the activities to regulate GLUT on erythrocyte membrane to inhibit glucose transportation, and depletion of glycogen in liver to release glucose into bloodstream (Kobayashi et al., 2000; Moreira et al., 2013). Moreover, it has been reported that catechins were also activate adipocytes in order to breakdown triglyceride to obtain intermediates for gluconeogenesis in the liver (Ueda et al., 2010). Hence, green tea extract had potential activity to regulate glucose homeostasis during malaria infection.

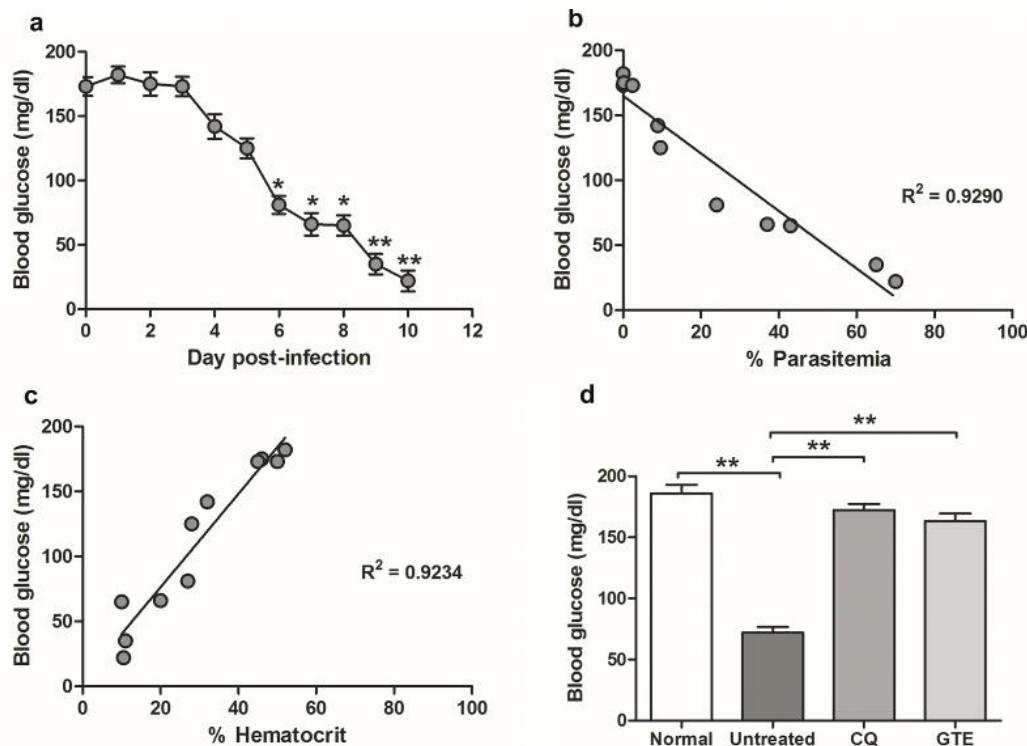


Figure 2. Efficacy of green tea extract on blood glucose levels during *Plasmodium berghei* ANKA infection. Groups of naïve ICR mice (5 mice/group) were inoculated with 1×10^6 parasitized erythrocytes of PbANKA by IP injection, and blood glucose levels were subsequently measured. (a) Blood glucose levels. (b) Correlation of blood glucose levels and parasitemia. (c) Correlation of blood glucose levels and hematocrit. (d) Efficacy of green tea extract on blood glucose during PbANKA infection on day 6. CQ, chloroquine treated group. GTE, green tea extract treated group.

* $p < 0.05$. ** $p < 0.01$.

4. Conclusion

Hypoglycemia has been reported previously in mice infected with *P. berghei*. However, this study appears to be the first report of hypoglycemia and efficacy of green tea extract to maintain blood glucose during infection with *P. berghei* ANKA. This may therefore be a good model in which to study the effect of green tea extract on glucose homeostasis during malaria infection *in vivo*.

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