Protection of renal function by green tea extract during Plasmodium berghei infection

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Abstract

Impairment of renal function from oxidative stress during malaria infection is one of the leading causes of death in endemic areas. Since blood urea nitrogen and creatinine levels in plasma can be used as markers for monitoring renal damage, this study investigated the effect of green tea extract on reduction of blood urea nitrogen and creatinine levels during malaria infection using Plasmodium berghei ANKA infected mice as in vivo model. For in vivo testing, ICR mice were infected with 1 × 10⁷ parasitized erythrocytes and green tea extract was subsequently administered orally twice a day for 10 consecutive days. Parasitemia was estimated by standard microscopy, and blood urea nitrogen and creatinine levels in plasma were also measured. It was found that parasitemia kept increasing until animal death, and was strongly correlated with high blood urea nitrogen and creatinine. The highest levels of blood urea nitrogen and creatinine in plasma were found on day 10 after infection. However, blood urea nitrogen and creatinine levels in plasma were reduced and decreased significantly (p < 0.01) in green tea extract treated mice, compared with untreated group. It can be concluded that green tea extract can protect and maintain renal function during malaria infection, and this extract can be developed for use as a supplement and combination therapy.

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1. Introduction

Malaria remains one of the world’s largest burdens of disease that is widespread in tropical and subtropical areas. It is a major public health problem in sub-Saharan Africa and an estimated 2.5 billion people are at risk; it causes 300–500 million infections and 1–3 million deaths every year, especially in children under five years of age [1]. It results from infection by parasites belonging to the genus Plasmodium. The asexual blood stage parasite infects the erythrocyte and is responsible for all of the symptoms and pathology associated with malaria. Malaria associated renal injury occurs between 1 and 4% of hospitalized adult patients and 10–20% of children under five years of age [2]. The pathogenesis of malaria associated renal injury is multifactorial and not well characterized, but hypothesis suggests the involvement of oxidative stress during malaria infection and damage to the vital organs especially renal organ [3]. For screening of renal function, increasing of blood urea nitrogen (BUN) and creatinine in plasma can be used as critical markers. This has prompted research towards the discovery and development of compounds to protect or reduce renal injury during malaria infection. In this respect, plant resources are potential targets for the research and development of alternative malarial drugs, as a supplement or used in combination with standard antimalarials.

The effective antimalarial activity of the two plant-based drugs, quinine and artemisinin, has generated much interest to explore other plant resources for their possible antimalarial efficacy [4]. Green tea (Camellia sinensis), originated in China, is a widely consumed beverage throughout the world. It has attracted large attention, recently, both in the scientific community and in the public opinion, for its pronounced health benefits towards a variety of disorders from cancer to weight loss [5]. It was suggested that activities of green tea polyphenols are mostly due to their powerful scavenging and antioxidant activity. Antioxidant tea components are reported to have beneficial protective effects against cancers and pathogenic microorganisms. It has been reported that green tea in both crude and pure substance extracts have properties that protect and reduce vital organ damage induced by oxidative stress [6]. BUN and creatinine levels in plasma were decreased when treated with green tea extract in mouse models [7]. According to this, the main focus of this study was to evaluate the efficacy of green tea extract on reduction of BUN and creatinine levels in plasma during malaria infection using Plasmodium berghei infected mouse model.
2. Materials and methods

2.1. Plant material

Fresh leaves of green tea (C. sinensis) were obtained at the Royal Project Shop, Chiang Mai, Thailand. A voucher specimen has been deposited in the Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. The plant material was air dried at room temperature and then powdered. Green tea crude extract was prepared using hot water method [8] and contained 60% of total polyphenols, >40% of EGCG, and <0.1% of caffeine by HPLC.

2.2. Animals

Pathogen free, 4 week old ICR mice weighing 30–35 g obtained from the National Laboratory Animal Center, Mahidol University, Bangkok were used in this study. They were housed in plastic cages with saw dust as beddings and given pellet diet (CP diet 082, Perfect Companion Company, Bangkok, Thailand) and water ad libitum. The mice were kept in 12 h day/12 h night cycle with 22–25 °C. Experiments were started in 4–5 week old animals. Permission and approval for animal experiments were ratified by the Animal Ethics Committee, Faculty of Medical Technology, Western University.

2.3. Parasite strain and infection of animal

P. berghei ANKA (PbANKA), a chloroquine-sensitive strain, was used in this study. Frozen parasite from stock was passaged at least once through ICR mice before experiments, and maintained by mechanical passage in mice. The progress of infection was assessed daily by conventional microscopic examination of Giemsa stained thin blood smear. The inoculum consisted of 5 × 10^7 PbANKA parasitized erythrocytes per ml. This was prepared by determining both percentage parasitemia and the erythrocyte count of the donor mouse and diluting the blood with normal saline solution in proportions indicated by both determinations. Naïve mouse was inoculated intraperitoneally with 0.2 ml of infected blood containing about 1 × 10^7 PbANKA parasitized erythrocytes.

2.4. Antimalarial drug

Standard antimalarial drug, chloroquine diphosphate salt (CQ) was used to study in vivo drug susceptibility of PbANKA. The drug was freshly prepared in distilled water (DW) and administered orally by gavage [9]. Drug doses, expressed in mg/kg of body weight, were adjusted at the time of administration according to the weight of each mouse. The doses were based on the ED90 of these drugs on PbANKA infected mice.

2.5. Measurement of hematocrit

In order to evaluate the number of erythrocytes from PbANKA infected mice, tail blood was collected and introduced into a heparinized microhematocrit tube. The end of tube was sealed with putty and centrifugation was subsequently performed at 10,000 g for 10 min before the percent packed erythrocyte of total blood volume (% Hct) was calculated.

2.6. Assessment of renal function

Tail blood was collected in the heparinized microhematocrit tube, and subsequently centrifuged at 10,000 g for 10 min. Plasma was then collected into a new 1.5-ml microcentrifuge tube and used for BUN and creatinine measurements. Levels of BUN and creatinine in plasma were measured using a commercial kit (BioSystem S.A. Costa Brava 30, Barcelona, Spain), according to the manufacturer’s instruction.

2.7. Efficacy test in vivo

The modified Peters’ 4-day test was used for in vivo testing [10]. Naïve ICR mice were inoculated by intraperitoneal injection with 1 × 10^7 PbANKA parasitized erythrocytes. The mice were randomly divided into 4 groups of 5 mice per group and treated for 10 consecutive days with 3000 mg GTE/kg body weight orally twice a day. Three control groups were used; the normal, uninfected, and infected controls were treated daily with DW, and the drug treated control was given a subcurative dose of CQ (7.5 mg/kg). On day 10 of the experiment, tail blood was collected from each mouse and then parasitemia and

Fig. 1. Impairment of renal function during P. berghei ANKA infection. (a) Parasitemia, (b) Hematocrit, and (c) survival of ICR mice infected with 1 × 10^7 parasitized erythrocytes by PbANKA. Renal function was assessed by (d) plasma blood urea nitrogen (BUN) and (e) creatinine estimated on different days after infection. Results represent the mean ± standard error of mean (SEM); *p < 0.01 compared with day 0.
hematocrit were calculated. Moreover, levels of BUN and creatinine in plasma were also subsequently measured.

2.8. Statistics

Statistical analysis of the data was performed using GraphPad Prism Software (GraphPad Software, Inc., US). A one way ANOVA test was used to analyze and compare the results at a 95% confidence level. Values of \( p < 0.01 \) were considered significant. Results were expressed as mean ± standard error of mean (SEM).

3. Results

3.1. Impairment of renal function during \( P. \) berghei ANKA infection

ICR mice were infected with PbANKA by intraperitoneal injection, and the course of parasitemia was determined. Parasitemia was first detectable on day 2 after infection (≤1%) and reached 50% on day 12 (Fig. 1a). In addition, decreasing of Hct levels in infected ICR mice was also observed (Fig. 1b). Moreover, with respect to the mortality, a death rate of 100% was observed until day 10 after infection (Fig. 1c). Next, we observed that plasma BUN and creatinine levels were markedly increased in infected ICR mice, and the highest levels were observed on day 10 after infection (Fig. 1d and e).

3.1.1. Effect of green tea extract on renal function of mice infected with \( P. \) berghei ANKA

ICR mice infected with PbANKA by intraperitoneal injection were treated orally twice a day with green tea extract (GTE) for 10 days, and plasma parameters of renal function were then measured. The effects of GTE on renal function of mice infected with PbANKA are shown in Fig. 2. The BUN and creatinine levels were increased significantly (\( p < 0.01 \) compared with normal group) by malaria infection, 2-fold (Fig. 2a–b, untreated group). After oral administration of GTE, plasma BUN and creatinine levels declined significantly (\( p < 0.01 \) compared with untreated group) (Fig. 2a–b, GTE treated group).

4. Discussion

In this study, we provide evidence that describes the effects of GTE to reduce BUN and creatinine levels in plasma during PbANKA infection. Since this parasite is sensitive to chloroquine, this drug was used as the standard antimalarial treatment. In addition, the choice of 4 week old mice for the study was done to avoid the effect of anemia in the old mice and the effect of physiological changes associated with aging that may be induced on the treatment outcome [11]. Impairment of renal function during malaria infection has been reported and it is an important life-threatening complication of malaria infection [12]. Propagation of malaria in vivo was observed by increasing parasitemia.

Moreover, during malaria infection in vivo, oxidative stress was occurred, subsequently destruction of erythrocytes was induced as shown by low levels of hematocrit [13]. The onset of renal injury in ICR infected mice came out on day 4 after infection, it was confirmed by the increase of plasma BUN and creatinine levels. In addition, the highest levels of BUN and creatinine in plasma were observed on day 10 after infection. It can be described that malaria associated renal injury is proposed to be a consequence of parasite adhesion and exacerbated immune response against oxidative stress products during infection [3]. Therefore, proinflammatory molecules and products of oxidative stress have a central role to the development of the pathogenesis of malaria associated renal injury. The extent of reactive oxygen species-induced oxidative damage can be exacerbated by decreased efficiency of antioxidant and cytoprotective defense mechanisms [2]. Moreover, modifications in the permeability of renal vascular endothelium decreased \( O_2 \) delivery to cells and tissues and contributed to increased hypoxic microenvironments [14].

The BUN and creatinine levels in plasma showed approximately a 2-fold, significant increase; however, GTE inhibited this increase. Hence, GTE had a positive effect on plasma BUN and creatinine abnormalities. It can be suggested that catechins, major components in green tea extract showed antioxidant and anti-inflammatory properties [15]. Treatment with green tea extract did not affect the normal mice. It has been reported that catechins in green tea extract increase total antioxidant capacity, especially glutathione peroxidase, catalase, and superoxide dismutase [16]. This study supports green tea extract as an effective dietary component during malaria infection. It can be concluded that green tea extract has potent antioxidant and anti-inflammatory properties to decrease BUN and creatinine induced by malaria infection. Hence, green tea polyphenols are useful supplements in the prevention and treatment of inflammation in malaria.

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References


