Parasitology International 62 (2013) 548–551

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

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

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ARTICLE INFO

Article history:
Received 23 July 2013
Accepted 19 August 2013
Available online 27 August 2013

Keywords:
Green tea extract
Plasmodium berghei
Renal function

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 1×10^7 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 is 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.

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 with a mortality that can reach up to 45% [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.

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http://dx.doi.org/10.1016/j.parint.2013.08.004

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

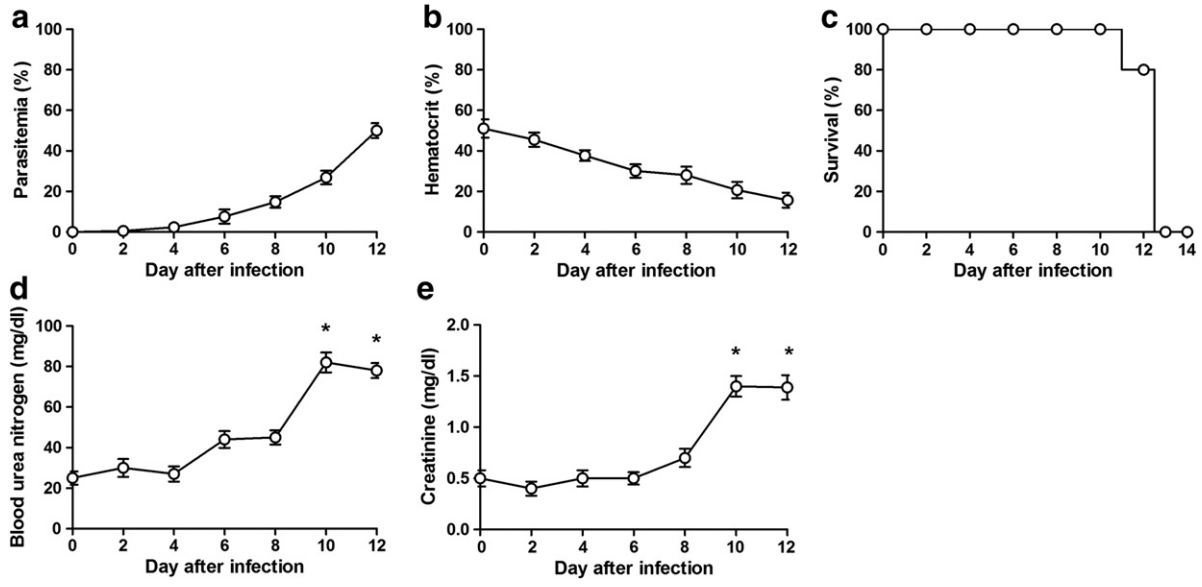

Fig. 1. Impairment of renal function during *P. berghei* ANKA infection. (a) Parasitemia, (b) Hematocrit, and (c) survival of ICR mice infected with 1×10^7 parasitized erythrocytes by PbANKA. Renal function was assessed by (d) plasma blood urea nitrogen (BUN) and (e) creatinine estimated on different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