J. Protozool. Res., 6, 46-51 (1996)

Hepatic Oxidative Stress and Antioxidant Defenses During Chloroquine-Resistant and Sensitive *Plasmodium berghei* Infection in Mice

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Received 31 October 1996 / Accepted 2 February 1997

Keywords: malaria, *Plasmodium berghei*, chloroquine-resistant, chloroquine-sensitive, oxidative damage, superoxide scavenging system.

ABSTRACT

Comparative study on hepatic oxidative stress and antioxidant defense systems of mice infected with chloroquine-resistant (CQ-R) or chloroquine-sensitive (CQ-S) *Plasmodium berghei* K173 strain revealed that *P. berghei* CQ-S strain infection makes the mice more vulnerable to oxidative damage as compared to *P. berghei* CQ-R strain infection. The levels of superoxide, lipid peroxidation, glutathione and xanthine-oxidase were significantly increased while, superoxide-dismutase and catalase showed a marked decrease during CQ-S *P.berghei* infection when compared to CQ-R *P.berghei*- infected host.

INTRODUCTION

Reactive oxygen species (ROS) play an important role in causing deterioration of biosystem. Free radicals particularly oxidant species such as superoxide anion, hydrogen-peroxide and hydroxyl radicals are implicated in the changes associated with a growing number of diseases (Pryor 1984). Active oxygen species are ubiquitous in living things, but the presence of an array of antioxidant defenses. Any alteration in the balance between the proxidant and antioxidant in the former side leads to oxidative stress or tissue damage (Sies 1985).

It has been observed that during parasitic infections, the levels of oxidative stress in the host increase, which can be detrimental to itself or the parasite (Halliwell and Gutteridge 1984). If the incurred oxidative damage is being

neutralized by the pathogenic organism (by anti-oxidant defenses) then it becomes easier for the survival of the parasite within the host or *vice-versa*. Malaria causes increased ROS generation in host, which may in turn affect the parasite. Clark et al (1984) have attempted a nonconventional chemotherapy by the production of increased ROS in host for eradication of malaria. This enhanced oxidative stress is however, normally encountered by the plasmodia allowing it to survive and possibly may share in the acquisition of resistance. Reports concerned with the above view are basically centered in depicting the status of plasmodia infected erythrocytes (Clark et al. 1989).

The present investigations are aimed to unveil the effect of *P. berghei* (CQ-R and CQ-S) infection on hepatic superoxide formation and it's scavenging system in mice with the view to find the role of host in helping the parasites in the acquisition of resistance.

MATERIALS AND METHODS

Experimental host and parasite

Colony bred albino mice (Swiss strain) weighing about 20-25 gm, free from infection and maintained in an air-conditioned room (temperature 25±1°C) were used. Mice were inoculated with either chloroquine-susceptible (CQ-S) or chloroquine-resistant (CQ-R) *P. berghei*-infected erythrocytes (Puri et al. 1979) and the parasitemia was monitored from day 3 post-infection in Giemsa strained blood smears.

Sample preparations

Animals of all sets were sacrificed and homogenization of liver carried out as previously reported (Srivatava et al. 1991). Livers from 3 matched animals was pooled for each set of analysis. Fractionation of homogenate was done by differential centrifugation; 600*g*, 10 min: supernate of post-nuclear fraction; 10,000*g*, 20 min: supernate of post-mitochondrial fraction; 105,000*g*, 60 min: cytosolic supernatant. All the procedures of tissue homogenization and fractionation were carried out at 0-4±1°C.

Assay Procedures

Superoxide anion was estimated following the appearance of adrenochrome, as a consequence of oxidation of epinephrine at 480 nm, using ex. coeff. 4020 $M^{-1}cm^{-1}$ (Green et al, 1955). Superoxide and lipid peroxidation (Utley et al. 1967) were measured in whole homogenate. Xanthine oxidase was assayed in the cytosolic fraction and the activity expressed in 10^{-5} μM of xanthine oxidized min^{-1} (mg protein) (McCord and Fridovich, 1960). Superoxide-dismutase (SOD) was

assayed in partially purified 11,000*g* supernatant (Kakkar et al. 1984). Activity was expressed as units (mg protein)$^{-1}$ being the amount of enzyme required to inhibit the rate of chromogen production by 50%. Catalase was assayed in whole homogenate (Sinha 1972). Glutathione level was measured at 412 nm (Meister and Anderson 1983) in whole homogenate after standard calibration. Glutathione-reductase (Racker 1955), glutathione-peroxidase (Milles 1959), and glucose-6-phosphate dehydrogenase (DeMoss 1955) were analyzed in the post-nuclear fraction (supernate of 600*g* fraction) using an ex. coeff. 6.22×10^6 $M^{-1}cm^{-1}$ in each case. Activity was expressed as units (mg protein)$^{-1}$. Protein was estimated according to the method of Lowry et al (1951) using bovine serum albumin as standard.

RESULTS

A comparative study on hepatic oxidative stress and anti-oxidant defenses was carried out during CQ-R and CQ-S *P. berghei* infection in mice. The results obtained showed a generalized trend of enhancement in the oxidative stress indices viz. superoxide anion, lipid peroxidation and xanthine-oxidase. On the contrary, the anti-oxidant defense indices viz. SOD and catalase were progressively decreased with increasing parasitemia during CR-Q and CQ-S *P. berghei* strain infection when compared to normal. Hepatic superoxide anion (25%) and lipid peroxidation (58%) registered a marked decrease, whereas SOD (76%) and catalase (39%) showed noticeable increase at peak parasitemia (>50%) during CQ-R *P.berghei* infection compared to CQ-S *P.berghei* infection in mice (Fig. 1).

Glutathione-oxidant defense system indices viz. Glutathione, glutathione peroxidase, glutathione-reductase and glucose-6-phosphate dehydrogenase were significantly increased with the rise in parasitemia during CQ-R and CQ-S *P. berghei* infection in mice when compared to normal. There was an enhancement of 16, 38, 15, and 13% in Glutathione, Glutathione-peroxidase, Glutathione-reductase and glucose-6-phosphate dehydrogenase at peak parasitemia (>50%) due to CQ-R *P.berghei* infection compared to CQ-S *P. berghei* infection in mice. (Fig. 1).

DISCUSSION

Increased oxidative stress and it's decreased defenses are the inevitable process during the pathophysiology of any disease. Several reports exist about the increased oxidative stress in plasmodia infected erythrocytes. Recently, we reported that during CQ-S *P. berghei* infection in mice (Srivastava et al. 1991), there is increased hepatic oxidative stress and decreased anti-oxidant defenses, however, the area remains almost barren in presenting the status of host hepatic

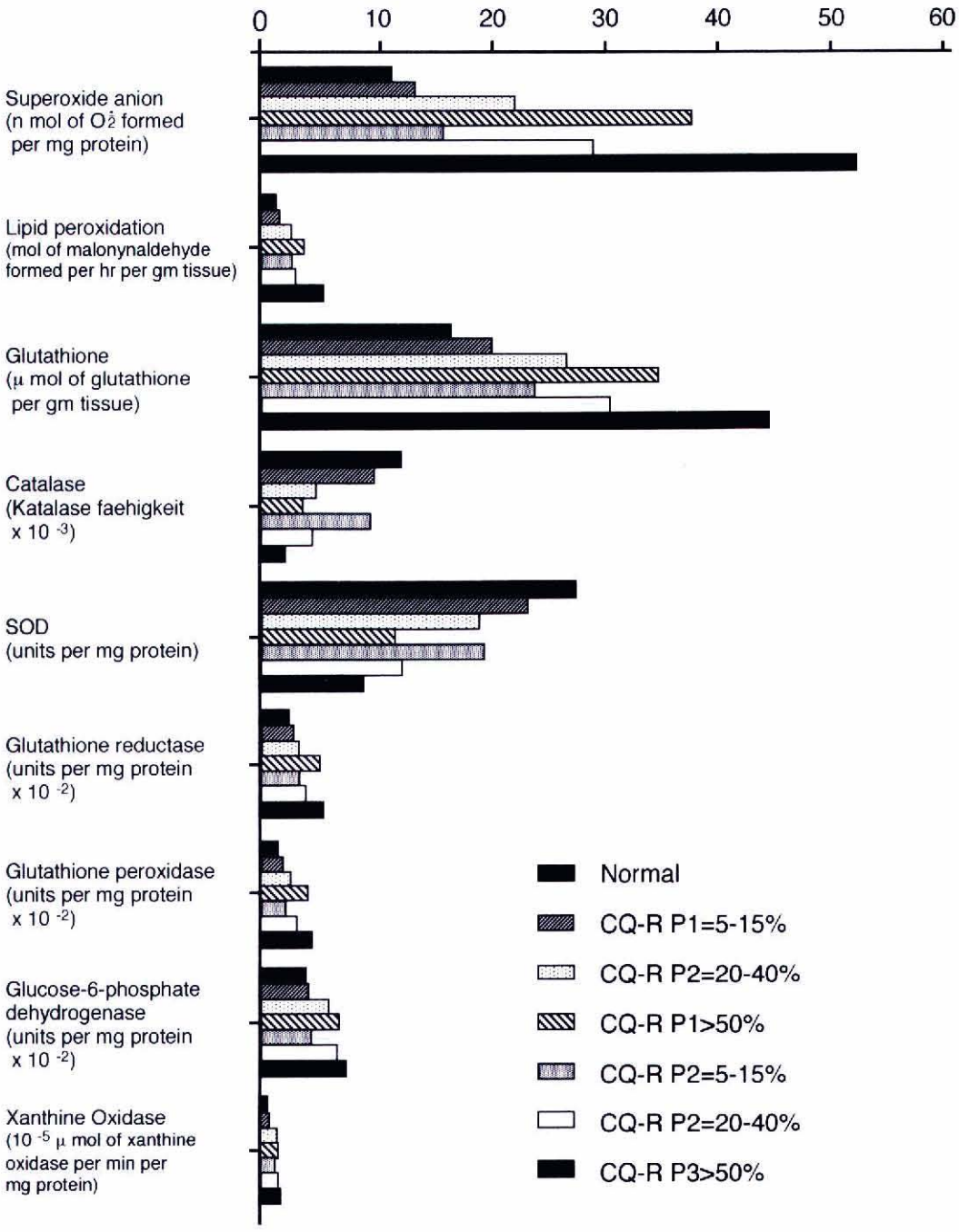

Fig. 1 A comparative study on hepatic oxidative stress and anti-oxidant defenses was carried out during CQ-R adn CQ-S *Plasmodium berghei* infection in mice.

superoxide generating and scavenging system during CQ-R *P. berghei* infection.

The present observations depict that CQ-R *P .berghei* strain infection makes the host less vulnerable to oxidative damage compared to it's susceptible counterpart. The decreased hepatic oxidative stress (superoxide, lipid peroxidation and xanthine-oxidase) and increased anti-oxidant defenses (SOD and catalase) during CQ-R *P. berghei* infection makes favorable condition for the better survival and propagation of resistant parasites. In other words, less ROS generation and more of it's dismutation allows the resistant parasites to survive in the host infected with CQ-S *P berghei* compared to it's susceptible counterpart.

The study further indicates that in case of resistant strain infected host, the parasite may release some factor which in turn cause activation of the host immune response (Jayshree et al. 1989), yielding in the elevation of anti-oxidant defense system, which may be essential for the better survival of the parasite. In fact, the resistant parasites alter the host metabolism according to their own requirement, so that they could thrive and propagate with ease, even in the presence of higher dossier of antimalarial treatment.

In conclusion, the authors emphasize that the host also plays a contributory role in maintaining the resistance of malarial parasites. The study could be helpful, if somehow or other, the alterations in the superoxide scavenging system due to CQ-R *P. berghei* infection may be equalized to it's susceptible counterpart, which in turn might make the resistant parasite vulnerable to ROS, resulting in an easy eradication by antimalarial treatment.

ACKNOWLEDGEMENT

Authors are thankful to director, CDRI for his keen interest in the study and to CSIR for providing Res. Assoc. PS. This is CDRI Communication number 4994.

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