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Glutathione-S-transferase activity in fractions parasitized by *Plasmodium berghei*

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Abstract: *Glutathione metabolism plays an important role in malaria infected red blood cells and parasite itself. Glutathione-S-Transferase (GST), an antioxidant enzyme plays vital role in protecting the parasite from host induced oxidative damage. During the present study, GST activity was found exhibit concomitant increase with increasing parasitaemia in the erythrocytes and as well as in cell free extract of Plasmodium berghei (NK-65), there by suggesting that erythrocytes are under oxidative stress. Results of present study showed 2 fold increase in specific activity of GST at parasitaemia i.e. 20% to 35% which increased to 2.5 fold at 55% parasitaemia. This study suggests the role of GST activity as novel candidate for biochemical markers in malaria.*

Key Words: *Glutathione-S-Transferase, Plasmodium berghei, malaria, parasitaemia*

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I. Introduction

Malaria is a complex vector borne disease afflicting all tropical regions of the world. It is a debilitating disease with serious socio-economic consequences, affects millions of people globally especially in eastern Africa and southeastern Asia. According to WHO, in the year 2016, there were 216 million cases of malaria worldwide with estimated 438,000 malaria deaths (WHO, 2017). Different investigators have studied biochemical make up of parasite as well as host tissues extensively. Glucose utilization through glycolytic pathway, Citric acid cycle and pentose phosphate pathway has extensively been studied by different researchers (Fulton and spooner, 1956; Bowman *et al*., 1960). However, most of the information about the enzymes pathways attributes to one or the other species of parasite, still the complete biochemical make-up of the parasite needs to be elucidated. The information on glutathione metabolism is scanty and exploration of this pathway may bring out new insights about the understanding of biochemistry of malaria parasite.

Malaria parasites produce oxidative stress in host erythrocytes, induces cell –mediated immunity (CMI), activation of phagocytes and soluble products of which being possibly the ultimate effector (Buffinton *et al*., 1986). The glutathione transferases (GSTs; E.C.2.5.1.18) are group of multifunctional enzymes, which plays important role in biotransformation of many xenobiotics by way of mercapturic acid pathway (Chasseaud, 1979). In addition to their role in catalyzing conjugation of electrophilic substrate to GSH they also have peroxidase and isomerase activities (Veal *et al.,* 2002). Many studies discussed the role of GST as a potential target of clinically used antimalarial drugs (Srivastava *et al*., 1999; Banyal and Sharma, 2007). In mice infected with *P. berghei*, reactive oxygen species (ROS) are produced (Eckman and Eaton, 1979). The production of increased amount of ROS reduces the antioxidant defence system viz. SOD, catalase, GR, GSHpx and glutathione.

In the present study, *P. berghei* has been used as research model because of its similarity in structure, physiology and life cycle with human malarial parasites along with similar molecular basis of drug sensitivity and resistance and easy *in vitro* and *in vivo* study.

II. Material and methods

Maintenance of the parasite

Plasmodium berghei (NK-65) was maintained in white Swiss mice, *Mus musculus* (BALB/c) as per guidelines of Institutional Animal Ethics Committee (IAEC) of Himachal Pradesh University, Shimla. The asexual erythrocytic stage of parasite was maintained by inoculation of 1 x 10^5 *P. berghei* - infected erythrocytes from the infected individual to the naive (Banyal *et al*., 1991).

Isolation of cell – free parasite and preparation of cell free parasite homogenate: Cell - free *P. berghei* (NK-65) was isolated by using 0.2% (w/v) saponin in 0.01M PBS, pH 7.2 (Kapoor and Banyal, 2011).

Preparation of Enzyme Extract

Normal and *P. berghei* infected erythrocytes, their membranes and cell free parasites were thawed. Erythrocytes and their membranes were suspended in citrate saline separately while cell-free parasites were suspended in phosphate buffer (0.015M, pH-7.2) and their homogenates were prepared by homogenizing them at 4^0C using Potter-Elvehjem homogenizer (Remi, Bombay). The prepared homogenates were centrifuged at 10,000 rpm for 30 minutes and supernatants were used for further study.

Treatment of Enzyme Extract

Before assay, enzyme extracts were incubated with phenyl methyl sulfonyl fluoride (PMSF, 0.1mM), a serine protease inhibitor dithiothreitol (DTT, 5.0mM), a reducing agent, overnight at 4^0C.

Enzyme Assay

Glutathione transferase (GST; E.C.2.5.1.18) was assayed by method of Habiq *et al*., (1974).

Protein Estimation

Protein concentration was estimated by slightly modified method of Lowry *et al.* (1951) using bovine serum albumen as standard.

III. Results

Parasite

NK-65 strain of rodent malaria parasite, *Plasmodium berghei* was found lethal to white Swiss mice *Mus musculus* (BALB/c) after inoculating $1x10^5$ *P. berghei*-infected erythrocytes to normal mice, the peak parasitaemia (35-55%) was observed by day 8 post inoculation.

Leucocyte Free Erythrocyte Preparation

The leucocyte free normal and *P. berghei* –infected erythrocytes were prepared by passing the blood through CF-11 cellulose column. Fig.1. shows the giemsa stained smear of *P. berghei*-infected blood before passing through CF-11 cellulose column containing a leucocyte. The blood smear of mouse after passing through CF-11 cellulose column showed asynchronous stages of the parasite i.e. rings, trophozoites and schizonts (Fig.2).

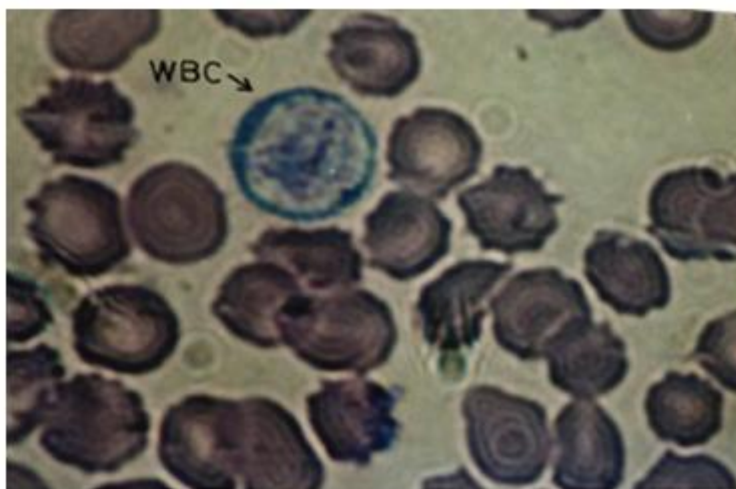

Fig.1: Blood Smear of *P. berghei* infected RBCs before passing through CF-11 Cellulose column showing a Leucocyte. **WBC- White Blood Corpuscle (Leucocyte)**

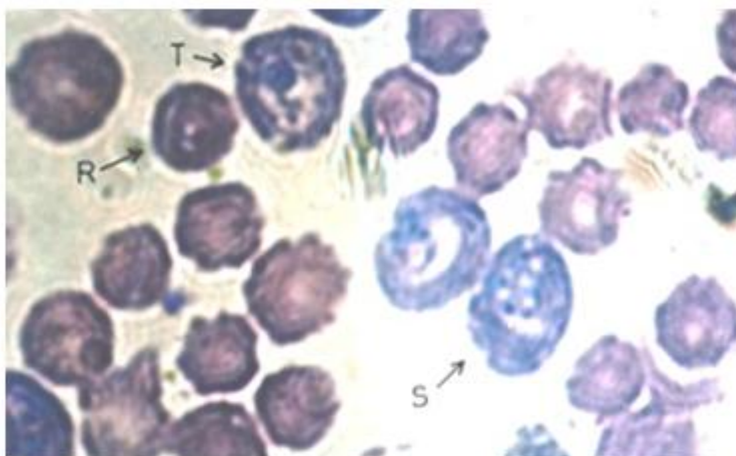

Fig.2: Blood Smear of *P. berghei* infected RBCs after passing through CF-11 Cellulose column showing asynchronous asexual erythrocytic stages of parasite. **R- Ring, T- Trophozoite, S- Schizont**

Glutathione Transferase Activity In Erythrocytes And Plasma

GST activity was evaluated in erythrocytes and plasma from normal and *P. berghei* - infected mice at different parasitaemia. Table 1 shows the specific activity of GST in erythrocytes with increasing parasitaemia. Two fold increase in specific activity of GST at parasitaemia 20% to 35% was observed, which increased to 2.5 fold at 55% parasitaemia. The specific activity was negligible in plasma at different levels of parasitaemia. The maximum increase in specific activity of GST was obtained at 55% parasitaemia. The GST activity was determined in various cellular fractions of infected erythrocytes as shown in table 2. The significant increase in GST activity was obtained in membrane fraction of infected erythrocytes and haemolysate.

Glutathione Transferase Activity In Cell-Free *P. berghei*

Table 3. shows that cell-free *P. berghei* possesses its own GST activity. Specific activity of 0.027±0.008 was observed.

Table 1. GST activity in erythrocytes and plasma from normal and *P. berghei*-infected mice at different parasitaemia.

Tissue	GLUTATHIONE TRANSFERASE Parasitaemia	Activity (U/ml)	Specific Activity (U/mg)
	Normal	0.262±0.10	0.013±0.005
	20%	0.162	0.013
Erythrocytes	35%	0.212±0.08	0.025±0.007
	55%	0.231±0.10	0.0035±0.003
	Normal	0.157±0.01	0.009±0.001
Plasma	20%	0.106	0.005
	35%	0.173±0.08	0.011±0.008
	55%	0.133±0.03	0.004±0.001

Results are mean ± S.D. of three different experiments each runs in duplicate except at 20% Parasitaemia.

Table 2. GST activity in 55% parasitized erythrocytes and their fractions.

Fractions	Activity (U/ml)	Specific Activity (U/mg)
Total erythrocytes	1.176± 0.16	0.013±0.008
Haemolysate	0.214± 0.08	0.029±0.007
Membranes	0.059± 0.10	0.126±0.008

Results are mean ± S.D. of three different experiments each runs in duplicate.

Table 3. GST activity in Cell-Free *P. berghei.*

Experiment	Activity (U/ml)	Protein (mg/ml)	Specific Activity (U/mg)
1.	0.229	6.053	0.037
2.	0.125	4.882	0.025
3.	0.135	6.737	0.020
Mean ±SD	0.163±0.05	5.90±0.93	0.027±0.008

IV. Discussion

In the present study, the level of GST in erythrocytic stages of *P. berghei* and also in cell-free parasite was determined. It has been observed in the present study that the GST activity increased with increasing parasitaemia. High GST activity in total infected erythrocytes of BALB/c mice indicates their susceptibility for oxidants. The increase in GST activity with increasing parasitaemia indicates that enzyme is actively the reaction of glutathione as a nucleophile with variety of compounds bearing an electrophilic centre. The haemolysate of *P. berghei*-infected erythrocytes has shown 0.029 units of specific activity, which was almost 2.2 times higher than the normal. Similar results of increased GST activity in chloroquine resistant *P. berghei* was observed by Dubious *et al*., 1995. This indicates that defence system for detoxification of reactive oxygen species in red blood cells is glutathione redox cycle.

During the infection of erythrocytes by the parasites, there is reduced level of glutathione, accompanied by lipid peroxidation, haemoglobin denaturation and formation of Heinz bodies (Brenner and Allison, 1953). Hence, during the parasite interaction there is activation of respiratory burst in leucocytes with production of superoxide ion and hydrogen peroxide. These reactive oxygen species imposes stress to erythrocytes, which further causes the oxidation of haemoglobin. This oxidized haemoglobin bound to the membranes indicates lipid peroxidation (Allison and Eugui, 1983).

The Present study supports this since increase in GST activity was observed with increasing parasitaemia in *P. berghei*-infected erythrocytes, which indicates that erythrocytes are under oxidative stress. The high GST activity was also determined in membrane fraction of infected erythrocytes. The activity of GST in pooled erythrocyte stage of *P. berghei* indicates the role of GST in parasite specific xenobiotic detoxification and further in antioxidant defence of parasite. The GST activity in blood stage of cell-free *P. berghei* was 0.163 U/ml. The parasitaemia dependent increase in GST can further be explained on the basis of facts that during plasmodium infection there is decreased life span of non-parasitized erythrocytes and enhanced splenic clearance of parasitized erythrocytes (Looareesuwan *et al.,* 1991). In intact trophozoite infected erythrocytes glutathione level is low while oxidized glutathione (GSSG) is higher in comparison to normal erythrocytes, which indicates that host cell is facing oxidative challenge. GSH and GST play crucial role in defence against oxidative stress and detoxification of various toxic compounds. Therefore, studies on glutathione system are of paramount importance in malaria and can further be exploited.

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