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Polyamine Metabolism in Various Tissues During Pathogenesis of Chloroquine-Susceptible and Resistant Malaria

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The pathophysiological impact of infections with chloroquine-susceptible (CQS) and chloroquine-resistant (CQR) strains of *Plasmodium berghei* in *Mastomys natalensis* was studied with respect to changes in polyamine profiles in various tissues. Both CQS and CQR infections produced similar changes in polyamine profiles of various tissues. Maximum increase was recorded in spleen followed by liver and lungs. Renal, cardiac and cerebral tissues did not register significant changes. An increase in spermidine level was more prominent as compared to putrescine and spermine, leading to an overall increase in spermidine/spermine ratio. This ratio is an important index of cellular proliferation. Liver did not show considerable change in the activities of ornithine decarboxylase and S-adenosyl methionine decarboxylase, the regulatory enzymes of the polyamine biosynthetic pathway. Spleen however, registered marked induction of both the enzymes which was more prominent in the CQS infection than CQR. Normal erythrocytes contained traces of polyamine while the erythrocytes loaded with *P. berghei* parasites exhibited appreciably higher polyamine levels. Spermidine was detected in about five-fold higher concentrations than putrescine and spermine which were detected in equimolar levels. Again, CQS as well as CQR *P. berghei*, exhibited qualitatively and quantitatively similar polyamine profiles thus ruling out a role of polyamines in CQ-resistance in malaria.

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KEY WORDS — malaria; *Plasmodium berghei*; polyamines; chloroquine; resistance

ABBREVIATIONS — ODC, ornithine decarboxylase; SAMDC, S-adenosylmethionine decarboxylase; CQS, chloroquine susceptible; CQR, chloroquine resistant

INTRODUCTION

Pathogenesis of malarial infection progresses with several biochemical, immunological and pathophysiological changes in the host.[1] Immunological and pharmacological mediators released during the malaria infection induce protective proliferative responses in many stress and target organs. Spleenomegaly, hepatomegaly,[2] pulmonary oedema,[3] cerebral hypoxia and lesions[2] have been particularly demonstrated during malaria infection. Polyamines namely, putrescine, spermidine and spermine play an important role in cellular proliferation.[4] Rapid induction of polyamine biosynthesis is one of the major responses of cells to growth-promoting stimuli.[5] Therefore polyamines may also have a significant role in responses of various tissues and organs to the stress induced by the pathogenesis of malaria infection. Resistance of the malarial parasite to classical antimalarials namely, chloroquine is one of the major problems encountered in the treatment of disease.[6] Apart from several molecular and

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biochemical differences in the chloroquine-susceptible and resistant strains of the parasites, some differential factors generated in the hosts during the course of infection may also be responsible for differences in response of the parasites to the drug treatment.[1] In view of these, the polyamine metabolism of various tissues has been evaluated during infection with chloroquine susceptible (CQS) and resistant (CQR) *Plasmodium berghei* in *Mastomys natalensis*, an established experimental model for the study of pathophysiology of malarial infection.

MATERIALS AND METHODS

Parasite and Host

Plasmodium berghei berghei maintained in colony-bred *Mastomys natalensis* (multimammate rats) (weighing 30–35 g) was employed in the present studies. One batch of these animals was inoculated with 1×10^6 erythrocytes infected with chloroquine-resistant (CQR) *P. berghei* developed by the relapse technique of Warhurst and Folwell[8] to tolerate 80 mg kg^{-1} body weight of chloroquine and which had been maintained during 80–100 passages. Simultaneously another batch of the animals was inoculated with chloroquine-susceptible (CQS) *P. berghei* parasites. The remaining animals served as the healthy controls.

Blood from normal and infected animals (parasitaemia 30 ± 5 per cent or as specified) was drawn from the eye vein (optical plexus) with a glass capillary and collected in pre-chilled acid-citrated-dextrose (ACD). Animals from all the batches were killed by cervical dislocation immediately after collecting the blood and then liver, spleen, kidney, brain, heart and lungs were excised. Any adherent tissue was removed and the excised samples washed with normal saline. If required they could be stored at $-70^{\circ}C$.

Analysis of Polyamines

The tissues and blood obtained from healthy controls and the animals infected with CQS and CQR *P. berghei* were immediately processed for the analysis of polyamines. A 20 per cent (w/v) homogenate of the tissues was prepared in cold perchloric acid (PCA) (0·3 M). The blood from control as well as malaria-infected animals, was centrifuged at 100 *g* for 10 min. Plasma and buffy coats were removed and the erythrocytes were washed three times with normal saline. Erythrocyte pellets, washed thoroughly, were finally resuspended in an equal volume of 0·3 M PCA and vortexed. The homogenates and blood samples in PCA were kept on ice at least for 1 h and then centrifuged at $1000 \times g$ for 15 min. Polyamines namely, putrescine, spermidine and spermine were analysed in the PCA supernatant by the technique of reverse-phase HPLC.[9] Precolumn derivatization of the sample was performed with benzoyl chloride, it was then extracted into chloroform, washed and finally evaporated over a stream of air. A mixture of methanol:water 60:40 served as the mobile phase for elution of the polyamines through a Bondpack C_{18} (Waters, U.S.A.) column.

Ornithine Decarboxylase (ODC) and S-Adenosylmethionine Decarboxylase (SAMDC) Assays

ODC and SAMDC were assayed in liver and spleen only because these are the primary target organs affected during pathogenesis of malarial infection. ODC was assayed radiometrically according to the method described by Hayashi and Kameji.[10] Briefly, 20 per cent (w/v) homogenates of the tissues were prepared in a medium containing 50 mM Tris (pH 7·2), 50 μM pyridoxal phosphate, 5 mM dithiothrietol and 0·1 mM ethylene diamine tetraacetic acid. The homogenates were centrifuged at 10,000 *g* and the supernatants were used for the ODC assay. Similarly, the SAMDC was also assayed radiometrically according to the method described by Pegg and Pöso.[11] Protein was assayed according to the method of Lowry *et al.*[12] using bovine serum albumin as the standard.

RESULTS

The gross analysis of fresh weights of the tissues from healthy control animals and also from the *P. berghei*-infected (CQS and CQR strains) groups of animals are presented in Figure 1 while Figures 2 and 3 depict polyamine profiles of these tissues and erythrocytes from the above group of animals. Since liver and spleen are the most affected organs during malarial infection, these tissues were also analysed for the activities of ODC and SAMDC which are the regulatory enzymes of the polyamine biosynthetic pathway. The activity profiles are presented in Figure 4. The observations recorded are presented herein taking the individual tissues into consideration.

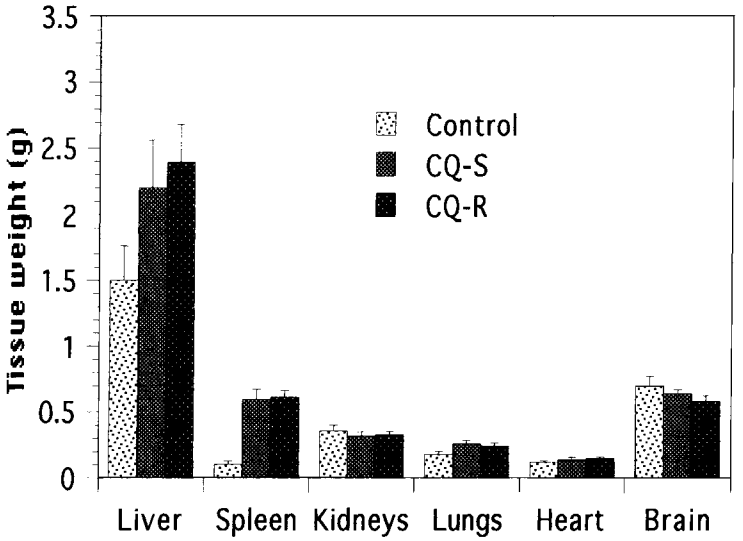

Figure 1. Comparative analysis of fresh weights of different tissues of *Mastomys natalensis* infected with CQS and CQR strains of *Plasmodium berghei*. Each bar represents mean value ± SD of at least three animals.

Liver

CQS as well as CQR *P. berghei* infection produced almost the same degree of hepatomegaly. In both the cases the gross weight of liver registered about a 1·5-fold increase (Figure 1). Similarly, both the infections resulted in almost identical changes in polyamine profiles. Spermidine was observed to be the major polyamine of hepatic tissues and registered the maximum increase (about 1·8-fold) due to *P. berghei* infection. Spermine levels were only marginally elevated while putrescine remained unaltered (Figure 2).

Spleen

Splenic tissues registered an approximately 5–6-fold increase in gross tissue weight, during both CQS as well as CQR infection (Figure 1). Levels of all the three polyamines namely, putrescine, spermidine and spermine were considerably elevated following *P. berghei* infection. Spermidine exhibited the maximum (three-fold) increase as compared to the other two polyamines (Figure 2). Again the changes were qualitatively and quantitatively similar during CQR and CQS infections.

Lungs

Gross examination of pulmonary tissues revealed haemorrhage consequent to *P. berghei* infection. However the fresh weight of the lungs was little changed (Figure 1). The spermidine level exhibited only about a 1·5-fold rise due to CQS as well as CQR *P. berghei* infection, while the other two polyamines namely, putrescine and spermine did not show much change due to the infections (Figure 1).

Kidneys, Heart and Brain

All three organs did not show noticeable change in gross weight during the infection (Figure 1). Renal as well as cardiac tissues exhibited a greater concentration of spermine than spermidine, while in the brain, spermidine was the major polyamine followed by putrescine. The polyamine profile of these tissues remained unchanged during infection except that a marginal rise in putrescine levels was detected in the brain.

Erythrocytes

Erythrocytes obtained from healthy control *M. natalensis* contained very low levels of polyamines. Intraerythrocytic proliferation of *P. berghei*, CQS and CQR parasites produced qualitatively and quantitatively almost similar rises in RBC polyamine levels. In *P. berghei*-infected erythrocytes, for both CQS as well as CQR, spermidine was observed to be the major polyamine and was present in almost five times greater concentration

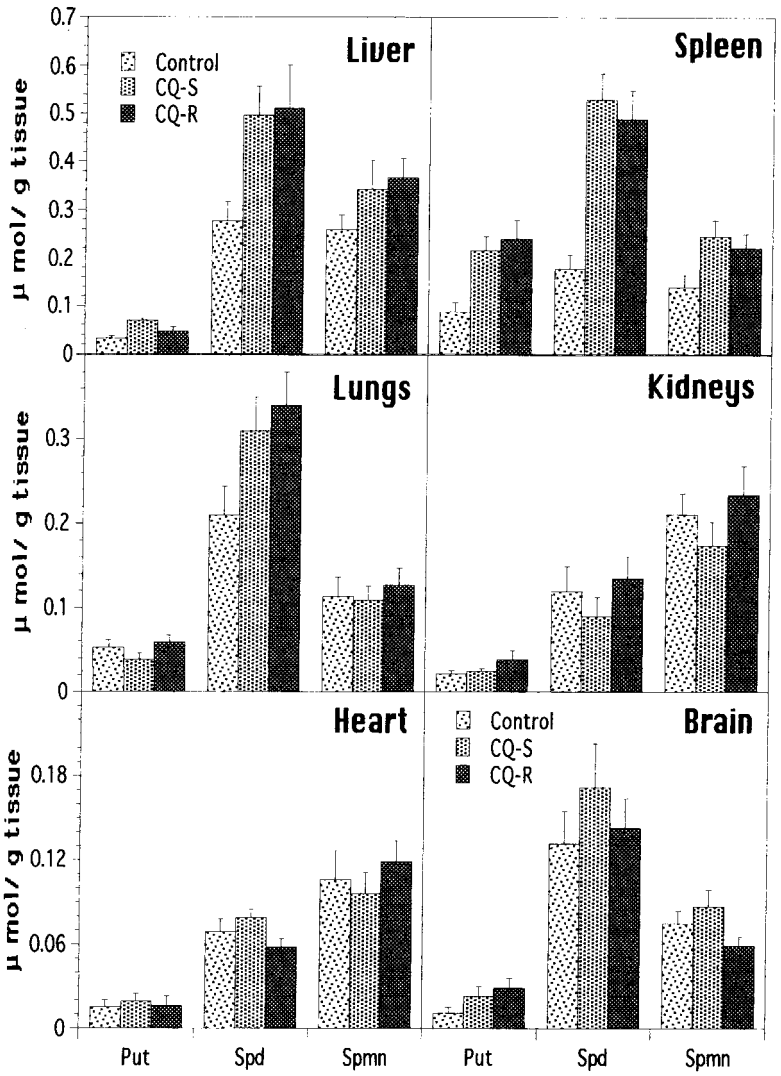

Figure 2. Polyamine profiles of various tissues of *Mastomys natalensis* infected with CQS and CQR strains of *Plasmodium berghei*. Each bar represents mean value ± SD of at least three observations from separate animals. Put, putrescine; Spd, spermidine; Spmn, spermine.

than putrescine and spermine. Both of the latter were detected in almost equimolar concentrations (Figure 3).

Activity of ODC and SAMDC

As already mentioned these enzymes were analysed in liver and spleen (Figure 4). A preliminary analysis of ODC activity during the course of malarial infection showed maximum change in the middle parasitaemia levels and becoming gradual at the peak level of parasitaemia. The comparative ODC and SAMDC profiles during pathogenesis of CQS and CQR. *P. berghei* infections were evaluated at mid-parasitaemia (15–20 per cent) levels only.

The liver did not show any marked change in the activity of either of the enzymes. ODC activity was marginally elevated in CQS *P. berghei*-infected liver while SAMDC was marginally induced in CQR-infected liver only. Spleen which undergoes great hyperplasia also revealed a marked induction of ODC as well as SAMDC activity. The induction was more prominent in CQS infection than during

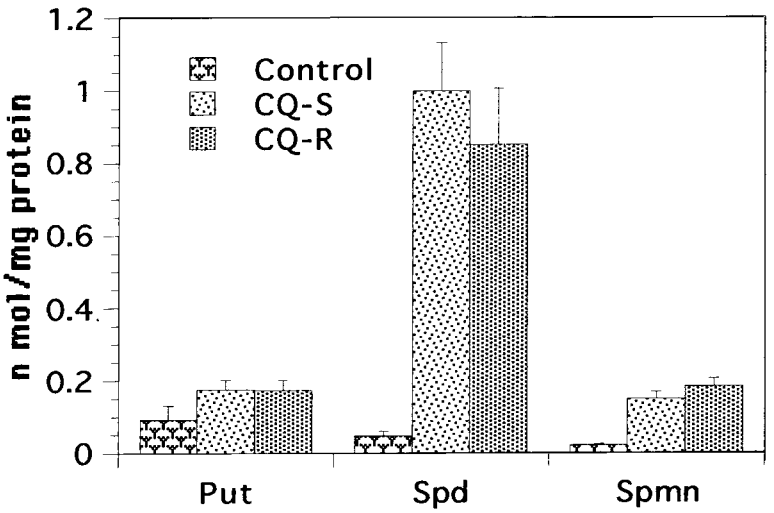

Figure 3. Polyamine profiles of erythrocytes from *Mastomys natalensis* infected with CQS and CQR strains of *Plasmodium berghei*. Each bar represents mean value ± SD of at least three observations from different animals. Put, putrescine; Spd, spermidine; Spmn, spermine.

CQR infection. No activity of ODC or SAMDC could be detected in *P. berghei*-infected erythrocytes employing the current protocols of assay.

Spermidine/Spermine Ratio

This ratio is an important index of cellular proliferation.[13] Figure 5 shows that liver, spleen and lungs showed considerable increase in this ratio. The increase in the spermidine/spermine ratio was maximal in spleen (CQS/CQR) (1·76/1·84) followed by lung (1·47/1·42) and liver (1·38/1·31). The values given in parentheses show that the increase in spermidine/spermine ratio was almost the same in CQS and CQR *P. berghei* infections. In kidney and heart this value remained unaltered, while in brain, a marginal increase was seen only in a case of brain infected with the CQR *P. berghei* strain.

DISCUSSION

The role of polyamines in proliferation of cells in response to various physiological, hormonal, immunological and other growth stimulii has been conclusively demonstrated.[5] The polyamine profile of any tissue/organ indicates the growth pattern of the tissue/organ. The results presented herein clearly indicate that infection with CQS and CQR strains of *P. berghei* induce similar levels of

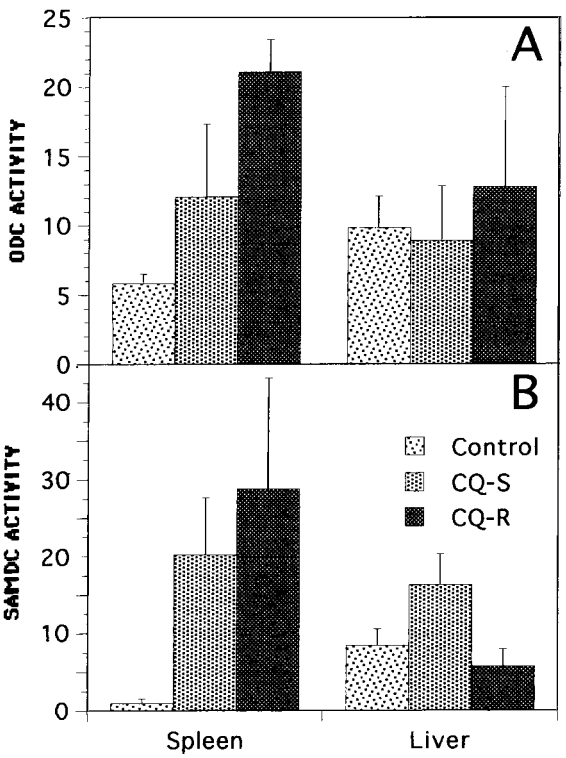

Figure 4. Ornithine decarboxylase (ODC) (A) and S-adenosylmethionine decarboxylse (SAMDC) (B) activity in liver and spleen of *Mastomys natalensis* during infection with CQS and CQR strains of *Plasmodium berghei*. Each bar shows mean value ± SD of at least three observations.

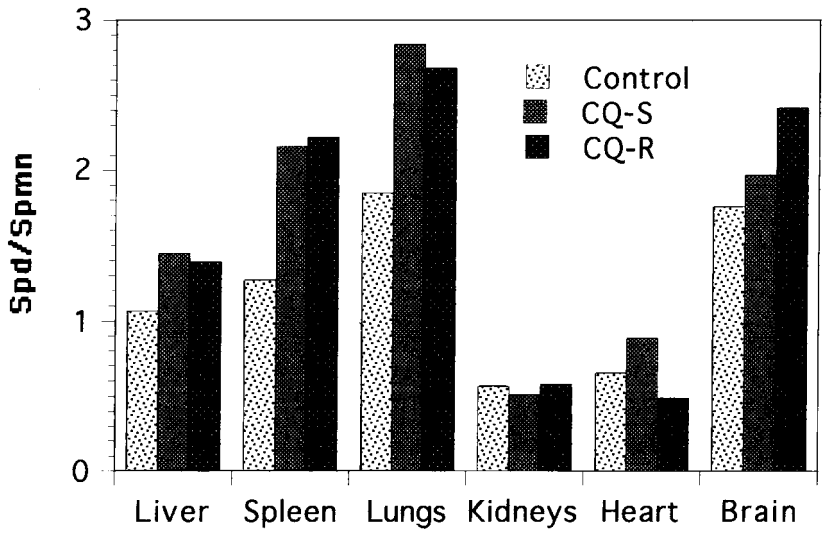

Figure 5. Spermidine/spermine ratio of different tissues of *Mastomys natalensis* infected with CQS and CQR strains of *Plasmodium berghei*. The ratios were computed from the mean values presented in Figure 2.

proliferative stress in various tissues as evaluated by the analysis of polyamine profiles. Liver and spleen are the main target organs during pathogenesis of malaria. Liver dysfunction mainly results from the sequestration of parasitized red cells in the hepatic and portal vasculature and from the accumulation of the pigment. This results in sinusoidal dilation with congestion of the centrilobular capillaries, swelling of hepatocytes, Kupffer cell hyperplasia, and infiltration of mononuclear cells.[2] All these responses should generate a prominent proliferative response in the hepatic tissues similar to that recorded during hepatic regeneration[14,15] and therefore show markedly high spermidine and spermine levels as compared to the normal tissue. Haematopoiesis and hyperplasia of spleen may be responsible for the marked increase in polyamine content of spleen during *P. berghei* infection. Similar observations have also been reported previously by Hibasani *et al.*[16] during infection of mice with *P. berghei*. Spleen registered an increase in putrescine together with a marked rise in spermidine and spermine contents which is due to marked induction of ornithine decarboxylase activity.

Pulmonary oedema is a common pathological feature observed during severe malarial infection.[3] This may possibly be the cause of the significant increase in spermidine level. Severe haemolytic anaemia during malarial infection causes hypoxia and it has been shown that polyamine biosynthetic activity is stimulated in lungs in response to hypoxia.[17] Infiltration of polymorphonuclear cells during malaria-induced pulmonary oedema and activation of alveolar macrophages may also be the reasons for the increased spermidine level[2] even though the lungs did not register a significant increase in tissue weight. Cerebral complications are mostly observed either during *P. falciparum* infection in humans[18] or in rodents in the case of a markedly high parasitaemia level e.g. *Plasmodium yoelii* infection in mice produced fatal severe cerebral complications.[19] It is pertinent to mention that *P. yoelii* has been reported to cause a decrease in putrescine and an increase in spermidine levels in cerebral tissues.[20] However, the degree of infection by *P. berghei* (CQS as well as CQR) tends to decline after reaching peak parasitaemic levels of 30 ± 5 per cent. Infection did not cause any significant change as evident from the results presented here. Similarly in renal and cardiac tissue the polyamine levels are not significantly changed indicating an unchanged proliferative index for these tissues.

Normal erythrocytes are anucleated and non-dividing cells and therefore polyamines are of doubtful physiological importance. The traces of polyamines detected in these cells may be accumulated from the surroundings during circulation as evidenced by the presence of distinct transport systems for polyamines in erythrocytes[21] which is markedly induced during intraerythrocytic proliferation of malarial parasites.[22] A marked elevation in polyamine profiles in the erythrocytes

loaded with *P. berghei* indicates the importance of polyamines in intraerythrocytic proliferation of the parasites. Similar polyamine profiles have earlier been reported in the erythrocytes infected with *P. falciparum*[23] and *P. knowlesi*.[24]

The results thus demonstrate that *P. berghei* infection produces marked induction of polyamine-mediated proliferation in liver and spleen that are qualitatively as well as quantitatively similar during pathogensis of CQS and CQR *P. berghei* infection. The presence of similar concentrations of polyamines in both CQS- and CQR-infected erythrocytes rules out a role of polyamines in the development of drug resistance in malaria.

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