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Status of Hepatic Mixed Function Oxidase System during Chloroquine-resistant and -sensitive *Plasmodium berghei* Infection in Mice

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ABSTRACT

Hepatic microsomal mixed function oxidase system was significantly inhibited/impaired during chloroquine-resistant and chloroquine-sensitive *Plasmodium berghei* K-173 strain infection in mice compared to normal. Cytochrome P-450, cytochrome b_5, aniline-hydroxylase, aminopyrine-N-demethylase and benzo(a)pyrene-hydroxylase were more affected due to chloroquine-sensitive as compared to chloroquine-resistant *P. berghei* infection in mice. Hepatic microsomal heme levels were increased during *P. berghei* infection as compared to normal but the increase was more in chloroquine-sensitive as compared to chloroquine-resistant *P. berghei* infection in mice.

INTRODUCTION

It is well established that mixed function oxidase (MFO) system plays an indispensable role in the clearance/metabolism of xenobiotics and endobiotics in living system (Kato 1977). It is involved in acquisition of resistance against drugs. Dearrangements in MFO system due to parasitic infection has been reported by many workers (Tekwani et al. 1987). Malaria infection is known to effect the MFO system of the host (Srivastava et al. 1991a, 1991b; McCarthy et al. 1980). So far the studies conducted related to MFO system dearrangements are with susceptible strains of plasmodia infection. With the growing resistance property of the malarial parasites, it has become interesting to know that whether the infection with chloroquine-sensitive and chloroquine-resistant *P. berghei* parasites display any dissimilarities in alteration of biochemical indices of the host. Presuming the

involvement of MFO system in imparting the resistance to the parasites, it was decided to monitor the status of hepatic microsomal MFO system during chloroquine-resistant and chloroquine-sensitive *P. berghei* infection in Swiss albino mice.

MATERIALS AND METHODS

Experimental Animals and Parasites

Colony bred Swiss albino mice, weighing about 18-20 g free from infection and housed in an air conditioned room (temperature 20±4°C) of the Animal House of Central Drug Research Institute were used. The animals were inoculated with 10^5 *Plasmodium berghei* K-173 strain (Chloroquine-sensitive and -resistant) (Puri et al. 1979) infected erythrocytes and the parasitaemia monitored from day 3 post infection in Giemsa-stained blood smears.

Sample Preparations

Animals of all groups were sacrificed and homogenization and fractionation of liver carried out as previously described (Srivastava et al. 1991).

Assay Methods

Enzyme assays were conducted as described by Srivastava et al. (1991). Protein was estimated according to Lowry et al. (1951) using bovine serum albumin as standard. p-values less than 0.05 were considered significant.

RESULTS

The data presented in Table 1 show a comparative account of hepatic mixed function oxidase (MFO) system during chloroquine-sensitive and resistant *Plasmodium berghei* infection in mice. The results obtained show a generalized trend of inhibition in the MFO-system indices viz. aniline-hydroxylase, aminopyrine-N-demethylase, benzo(a) pyrene-hydroxylase, cytochrome b_5 and cytochrome P-450, whereas, microsomal heme content was enhanced during infection when compared to normal. However, the extent of impairment of MFO system was more in case of chloroquine-sensitive as compared to chloroquine-resistant *P. berghei* strain infection in mice. Cytochrome P-450, cytochrome b_5, aniline-hydroxylase, aminopyrine-N-demethylase and benzo(a) pyrene-hydroxylase were inhibited by 30-50% whereas heme content was increased by >40% in chloroquine-sensitive *P. berghei* infection when compared to chloroquine-resistant infection at highest parasitaemia (>40%).

Table 1 Status of hepatic microsomal mixed function oxidase system during chloroquine-resistant (CQ-R) and chloroquine-sensitive (CQ-S) *Plasmodium berghei* infection in mice.

Parameters	Normal	(CQ-R) *Plasmodium berghei*			(CQ-S) *Plasmodium berghei*		
		12-15%	20-45%**	>50%***	12-15%	20-45%**	>50%***
Cytochrome P-450 [a]	1.11±0.10	0.68±0.11**	0.44±0.17	0.22±0.04	0.52±0.14**	0.30±0.07	0.11±0.01
Cytochrome b_5 [a]	0.57±0.07	0.50±0.10**	0.41±0.11	0.34±0.01	0.46±0.04**	0.36±0.08	0.32±0.13
Aniline hydroxylase [a]	56.41±3.12	49.04±3.41**	41.18±4.23	30.00±1.28	44.13±4.81**	31.18±4.26	27.43±3.21
Aminopyrine-N-demethylase [a]	1.54±0.19	1.12±0.07 *	0.62±0.11	0.39±0.07	0.74±0.08 *	0.47±0.14	0.32±0.06
Benzo(a)pyrene hydroxylase [b]	0.36±0.04	0.29±0.06 *	0.13±0.00	0.07±0.01	0.19±0.06 *	0.10±0.07	0.04±0.00
Heme [a]	2.45±0.29	3.65±0.23**	5.14±0.16	8.36±2.11	4.92±1.24**	7.08±0.37	10.14±1.39

Activities are expressed as [a] nmoles of product formed/mg protein; [b] change in relative fluorescence/mg protein.
Values are Mean ± S.D. of 6 separate observations from different animals.
* nonsignificant with normal; ** P<0.01-0.005; *** P<0.002-0.001

DISCUSSION

The present study demonstrated that chloroquine-sensitive and chloroquine-resistant *P. berghei* infection change the behavior of hepatic drug metabolizing system of the host. Sequential studies also maintain the usual profile of respective inhibition of host MFO system. At present it seems difficult to conclude that to what extent decreased inhibition of MFO system in chloroquine-resistant *P. berghei* infected host participate in providing protection to the parasite from drug pressure. However, it is certainly definite that less inhibition of MFO system will provide less opportunity to the drug to affect the parasite compared to the one whose or where the MFO system is more affected or inhibited.

The significant elevated level of hepatic heme in chloroquine-sensitive compared to chloroquine-resistant *P. berghei* infection probably further strengthen the views of Orjih and Fitch (1993) that chloroquine will get less opportunity to form ferriprotoporphyrin IX-chloroquine complex in case of chloroquine-resistant compared to chloroquine-sensitive *P. berghei* infection. It is also known that less inhibition in mixed function oxidase system is favorable in maintain the resistance imparted by host, as heme is also known to inhibit mixed function oxidase system. The authors feel that concomitant with the development of anti-resistant compounds, it will be better that cytochrome P-450 suppressive agents, should be incorporated for synergistic effect/impact on the resistant parasite.

In conclusion, the authors feel that the role of host MFO system can not be oversighted in acquisition of resistance of the parasite.

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