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Plasmodium berghei (ANKA): Infection induces CYP2A5 and 2E1 while depressing other CYP isoforms in the mouse liver

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Abstract

It has been reported that malaria infection impairs hepatic drug clearance and causes a down-regulation of CYP-mediated monooxygenase activities in rodents and humans. In the present study, we investigated the effects of *Plasmodium berghei* infection on the activity of liver monooxygenases in female DBA/2 and C57BL/6 mice. In both mouse strains, *P. berghei* infection decreased activities mediated by CYP1A (EROD: DBA/2 65.3%, C57BL/6 44.7%) and 2B (BROD: DBA/2 64.3%, C57BL/6 49.8%) subfamily isoforms and increased activities mediated by 2A5 (COH: DBA/2 182.4%, C57BL/6 148.5%) and 2E1 (PNPH: DBA/2 177.8%, C57BL/6 128.5%) isoforms as compared to non-infected controls. Since malaria infection also produced an increase in ALT (273.1%) and AST (354.1%) activities in the blood serum, our findings are consistent with the view that CYP2A5 activity is induced by liver injury. An almost generalized depression of CYP-mediated activities has been found with numerous infections and inflammatory stimuli but an induction of CYP2A5 had been previously noted only in some viral hepatitis and trematode (liver fluke) infections.

Index Descriptors and Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BROD, benzyloxi-resorufin-*O*-debenzylase; COH, coumarin-7-hydroxylase; CYP, cytochrome P450; EROD, ethoxyresorufin-*O*-deethylase; LPS, lipopolysaccharide; Monooxygenases; NO, nitric oxide; iNOS, inducible nitric oxide synthase; MAL, malaria-infected mice; CON, non-infected control mice; PNPH, *p*-nitrophenol-hydroxylase; Pharmacokinetics; *Plasmodium berghei* (ANKA); Xenobiotic metabolism; SEM, standard error of the mean

1. Introduction

Several clinical and experimental studies have demonstrated that malaria infection down-modulates the expression and activity of CYPs thereby decreasing the hepatic metabolism of drugs and other xenobiotic compounds in humans and rodents. In 1970, McCarthy and coworkers reported that infection of rats with *Plasmodium berghei* increased hexobarbital sleeping time and reduced hepatic metabolism of ethylmorphine, aniline and *p*-nitroanisole. Further studies found that infection with *P. berghei* decreased total-CYP content and monooxygenase activities in rat liver microsomes (Kokwaro et al., 1993; Uhl et al., 1999) as well as the clearance of xenobiotics such as artheether (Leo et al., 1997), ethoxyresorufin (Glazier et al., 1994), antipyrine (Mansor et al., 1991a), paracetamol (Mansor et al., 1991b), and phenacetin (Kokwaro et al., 1993). Malaria infection was found to reduce total-CYP content and monooxygenase activities in mouse liver as well (Alvares et al., 1984; Srivastava and Pandey, 2000; Srivastava et al., 1991, 1997).

Data from clinical pharmacokinetic studies have also been consistent with the hypothesis that malaria infection depresses CYP-mediated drug metabolism. It was reported, for instance, that clearances of quinine, antipyrine and caffeine were reduced in patients with acute *Plasmodium falciparum* malaria (Akinyinka et al., 2000; Pukrittayakamee et al., 1997).

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Contrasting to the foregoing findings suggesting that malaria causes a generalized depression of liver CYP activities, Uhl et al. (1999) reported that CYP2E1 activity remains unaltered in liver microsomes of *P. berghei*-infected rats. As far as the authors are aware, however, there has been no report of induction of liver monooxygenases by malaria infection so far.

In the present study, we investigated the effects of *P. berghei* infection on mouse liver monooxygenase activities mediated by CYP2A5 (COH) and 2E1 (PNPH) as well as by isoforms belonging to subfamilies CYP1A (EROD) and 2B (BROD). CYP1A1/2 and 2E1 are involved in the metabolic activation of a number of xenobiotic compounds including many chemical carcinogens (Ioannides and Lewis, 2004). Isoenzymes belonging to CY2B subfamily also take part in the activation of toxicants as well as in the metabolic clearance of artemisinin, an important antimalarial drug (Simonsson et al., 2003). It is of note that CYP2A5, a murine isoform orthologous to human CYP2A6, plays an essential role in the activation of various hepatotoxins and carcinogens, including nitrosamines, and apparently has a unique mechanism of regulation (Gilmore et al., 2003). Contrasting to most CYP isoforms, CYP2A5 is induced in some infectious diseases such as viral hepatitis and liver fluke infection (Kirby et al., 1994a,b).

2. Material and methods

2.1. Animals

Female C57BL/6 and DBA/2 mice, 7–10 weeks old, from the Oswaldo Cruz Foundation (FIOCRUZ) breeding stock were used. The animals were housed in standard plastic cages with stainless steel coverlids and wood shavings as bedding. Room temperature (23 ± 2 °C), air relative humidity (approx. 70%) and light/dark cycle (lights on from 8:00 am to 8:00 pm) were controlled in the animal facilities. Filtered tap water and a commercial diet for rats and mice (Nuvital CR1, Nuvilab®, Curitiba, PR, Brazil) were provided ad libitum. The experiments were conducted in accordance with Brazilian animal protection and welfare laws and the protocol was submitted to the Ethics Committee on the Use of Animals of Oswaldo Cruz Foundation.

2.2. Parasite and infection

Experimental animals were injected by the i.p. route with 0.2 ml of PBS-diluted blood (5×10^6 parasitized red blood cells per milliliter) from a donor mouse that had been previously infected with *P. berghei* (ANKA). The donor mouse was inoculated with a stabilate kept at −80 °C in Alsever's solution. For each malaria-inoculated mouse, an age-paired non-infected control animal was injected with 0.2 ml of PBS solution. After infection, mice were daily examined for clinical signs of illness and, every other day, a small drop of blood was taken from the tail tip for determination of the level of parasitemia. The percentage of parasitized red blood cells (parasitemia) was determined by microscopic examination of thin blood smears stained by the Romanowsky's method (Panóptico Rápido, Laborclin®, Pinhais, PR, Brazil). Infected mice (MAL) and their age-paired non-infected controls (CON) were killed by cervical dislocation during the second or third week after infection when parasitemia rose to levels equal to or higher than 20%.

2.3. Preparation of liver microsomal fraction

After death, livers were removed as quickly as possible, freed from fat and extra tissue, weighed and frozen at −80 °C until further use. Hepatic microsomes were prepared essentially as described by De-Oliveira et al. (1999) except by the use of Tris (100 mM)–KCl (150 mM) buffer (pH 7.4) instead of sacarose solution. Microsomal protein concentration was measured by using Bradford's reagent and bovine serum albumin as standard (Sigma Chemical Co, USA).

2.4. Enzyme assays

Activities of alcoxyresorufin-*O*-dealkylases were determined using benzyloxy-(BROD) and ethoxyresorufin (EROD) as substrates. Enzyme activities were determined in multiwell plates as described by Kennedy and Jones (1994), except for the use of a NADPH regenerating system as previously reported by De-Oliveira et al. (1999). Substrate concentration was 5 μM and 0.025 mg of microsomal protein was added to each well. After a 10-min incubation at 37 °C, 100 μL of acetonitrile was added to each well and the amount of resorufin was measured by using a fluorescence plate reader (Spectramax Gemini XS®, Molecular Devices Co, USA) with excitation and emission wavelengths set at 530 and 590 nm, respectively. Under the present assay conditions, reaction was previously found to be linear for at least 15 min.

COH activity was assayed as reported by Rhanasto et al. (2003) with a few adaptations. Coumarin (10 μM), Tris buffer, pH 7.4 (50 mM), microsomal protein (0.08 mg) were added to each well (final volume of 0.1 ml) and incubated at 37 °C with shaking for 10 min. Reaction was stopped with 60 μL of trichloroacetic acid and 140 μL of a 1.6 M glycine-NaOH (pH 10.4) solution was added to each well before measuring the amount of umbelliferone (7-hydroxycoumarin) by using a fluorescence plate reader with excitation wavelength set at 355 nm and emission at 460 nm. Under these conditions, reaction was found to be linear for more than 15 min.

PNPH activity in the mouse liver microsomal fraction was determined by the real-time kinetic method described in details by Allis and Robinson (1994). Substrate (*p*-nitrophenol) concentration was 0.1 mM and 0.2 mg of microsomal protein was added to each cuvette where reaction took place.

Serum ALT and AST activities were determined by a colorimetric method using a commercially available kit