Biochemical Pharmacology, Vol. 46, No. 10, pp. 1859–1860, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

SHORT COMMUNICATIONS

Effect of the antimalarial agents primaquine and (*N'*-3-acetyl-4-5-dihydro-2-furanyl)-N^4-(6-methoxy-8-quinolinyl)1,4-pentane-diamine on oxidative stress and antioxidant defences in mice

(*Received* 4 *May* 1993; *accepted* 12 *August* 1993)

Abstract—The effects of the newly developed antimalarial compound, CDRI 80/53 [(*N'*-3-acetyl-4-5-dihydro-2-furanyl)-N^4-(6-methoxy-8-quinolinyl)1,4-pentane-diamine], and primaquine (PQ) on the antioxidant system of mice were determined at equi-effective antimalarial doses on enzyme systems responsible for protection against oxygen, i.e. hepatic superoxide dismutase and catalase. While PQ significantly inhibited these enzyme activities CDRI 80/53 did not. However, both compound 80/53 and PQ increased the level of superoxide anion and lipid peroxidation. It is concluded that compound 80/53 has less effect on antioxidant defence enzymes than PQ.

Malaria increases reactive oxygen species (ROS*) and causes a decrease in the antioxidant defences of the host [1, 2]. Most antimalarials induce oxidative stress [3] and some kill the plasmodia by increasing ROS production [3]. These ROS besides being detrimental to the parasite can be hazardous to the host. An ideal antimalarial should therefore generate less ROS and interfere minimally with antioxidant defences. A new antimalarial currently undergoing Phase I clinical trial, the CDRI compound 80/53 [(*N'*-3-acetyl-4-5-dihydro-2-furanyl)-N^4-(6-methoxy-8-quinolinyl)1,4-pentane-diamine] (Fig. 1), is an enamine analogue of primaquine (PQ). Of CDRI 8.75 mg/kg body wt and of primaquine 7.00 mg/kg body wt cure sporozoite-induced *Plasmodium cynomolgi* B infection in rhesus monkeys [4]. However, CDRI 80/53 was less toxic than PQ, on the basis of met-hemoglobin formation [5], hepatotoxicity [6], and inhibition of aniline hydroxylase and aminopyrine-*N*-demethylase [7].

In the present study the effect of CDRI 80/53 on hepatic oxidative stress and the antioxidant defence system of mice is compared with that of PQ.

Materials and Methods

Swiss albino mice weighing 20–25 g were divided into five groups of 12 animals each receiving orally 80/53 at 8.75 (low dose) and 26.25 (high dose) mg/kg body wt, and PQ at 7 (low dose) and 21 (high dose) mg/kg body wt.

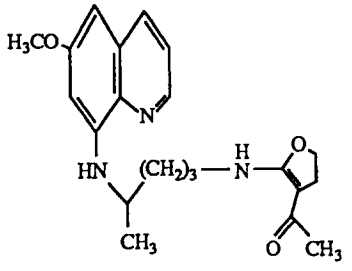

Fig. 1. Structure of CDRI antirelapse–antimalarial compound 80/53.

The fifth group remained untreated. After 4 days of treatment, half the mice were killed, and the other half were killed on day 8 by cervical dislocation. The livers were excised, homogenized and fractionated as reported earlier [1]. Superoxide anion ($O_2^{\cdot-}$) was measured by the formation of adrenochrome [8] and expressed as nmoles of $O_2^{\cdot-}$ formed per min per mg protein. Lipid peroxidation was analysed by malonyl dialdehyde formation at 535 nm [9]. Superoxide dismutase (SOD) was assayed in partially purified 11,000*g* supernatant [10], the activity being expressed as units per mg protein. Catalase was assayed according to the method of Sinha [11]. Protein was estimated according to the method of Lowry *et al.* [12] using bovine serum albumin as standard. The data were analysed for statistical significance using Student's *t*-test and ANOVA.

Results and Discussion

Primaquine, despite causing a range of side effects, is at present the drug of choice for treating falciparum and related malaria. A potential antirelapse–antimalarial compound 80/53 has been developed by the Central Drug Research Institute. While feeding of 80/53 for 3 days at the low dose had no effect on SOD activity, and decreased catalase activity by 35%; PQ at the low dose caused significant inhibition of SOD (22%) and catalase fell by 40% compared to control mice. Higher doses of PQ and 80/53 resulted in greater inhibition of the antioxidant defence system indices (Fig. 2c and d).

Figure 2a and b show that there was significant enhancement in hepatic $O_2^{\cdot-}$ and lipid peroxides levels after feeding compound 80/53 at both low and high dose compared to control. However, PQ induced higher levels of superoxide and lipid peroxides. Summerfield and Tudhope [13] demonstrated *in vitro* formation of $O_2^{\cdot-}$ with PQ. Studies from our laboratory showed that met-hemoglobin toxicity was three to four times lower with compound 80/53 compared to PQ [5], and the CDRI compound 80/53 is potentially an antirelapse–antimalarial drug.

Acknowledgements—The authors are indebted to Dr V. P. Kamboj, Director, CDRI for his keen interest in this work and to CSIR, New Delhi for providing SRF to the first author (P.S.). This is CDRI communication No. 5002.

Divisions of Biochemistry and ‡*Microbiology*
Central Drug Research Institute
Lucknow, 226 001
India

PRATIMA SRIVASTAVA†
S. K. PURI‡
G. P. DUTTA‡
V. C. PANDEY

* Abbreviations: PQ, primaquine; SOD, superoxide dismutase; $O_2^{\cdot-}$, superoxide anion; ROS, reactive oxygen species.

† Corresponding author.

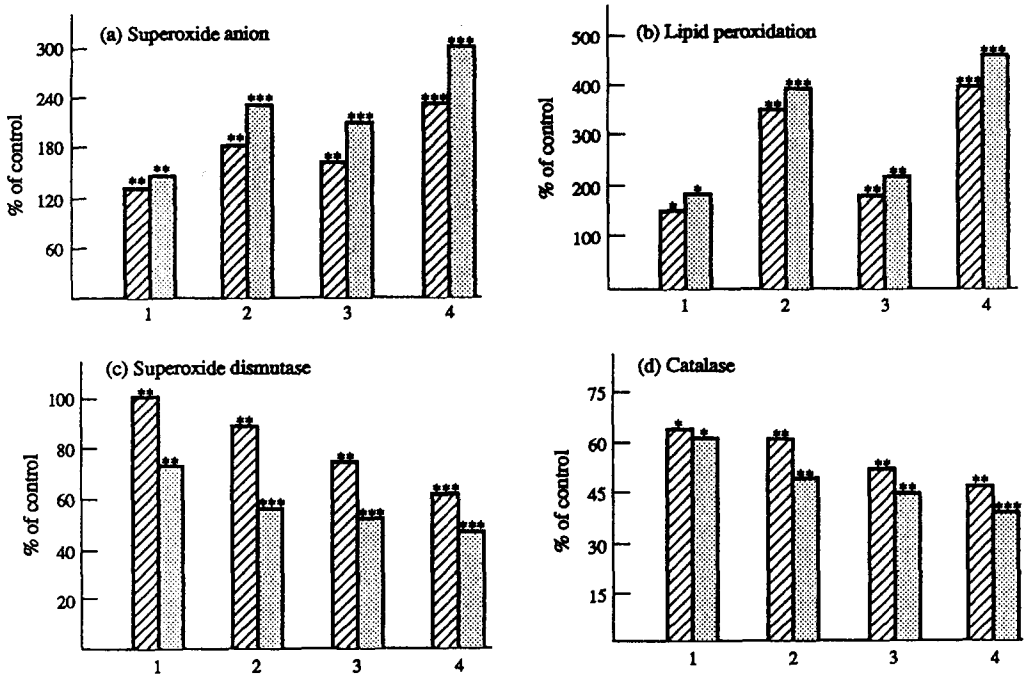

Fig. 2. Effects of compound 80/53 (▨) and PQ (▩) at (1) low dose, for 3 days; (2) low dose, for 7 days; (3) high dose, for 3 days; (4) high dose, for 7 days. Values are means of six separate observations from different animals. Statistical analysis was by ANOVA and multiple range testing. *Difference not significant with respect to normal; **$P < 0.01$; ***$P < 0.002$.

REFERENCES

1. Srivastava P, Puri SK, Dutta GP and Pandey VC, Hepatic superoxide-scavenging system during *Plasmodium berghei* infection and chloroquine treatment. *Med Sci Res* **19**: 307–308, 1991.
2. Srivastava P, Puri SK, Dutta GP and Pandey VC, Status of oxidative stress and antioxidant defences during *Plasmodium knowlesi* infection and chloroquine treatment in *Macaca mulatta*. *Int J Parasitol* **22**: 243–245, 1992.
3. Clark IA and Cowden WB, *Antimalarials: Oxidative Stress* (Ed. Sies H), pp. 131–185. Academic Press, New York, 1985.
4. Dutta GP, Puri SK, Bhaduri AP and Seth M, Radical curative activity of a new 8-aminoquinoline derivative CDRI 80/53 against *P. cynomolgi* B in monkeys. *Am J Trop Med Hyg* **41**: 635–637, 1989.
5. Puri SK, Srivastava R, Pandey VC, Sethi N and Dutta GP, Methemoglobin toxicity and hematological studies on malaria anti-relapse compound CDRI 80/53 in dogs. *Am J Trop Med Hyg* **41**: 638–642, 1989.
6. Pandey VC, Puri SK, Sahni SK, Srivastava P and Dutta GP, Effect of anti-relapse antimalarial compound CDRI 80/53 and primaquine on hepatic mixed function oxidase system of rhesus monkeys. *Pharm Res* **22**: 701–707, 1990.
7. Srivastava P, Sahni SK, Tripathi LM, Puri SK, Dutta GP and Pandey VC, Kinetic and substrate binding characterization of hepatic mixed function oxidase system in monkeys with primaquine and (*N'*-3-acetyl-4-5-dihydro-2-furanyl)-N^4-(methoxy-8-quinolinyl) 1,4 peptane-diamine. *Biochem Pharmacol* **43**: 904–907, 1992.
8. Green S, Mazur A and Shorr E, Mechanism of the catalytic oxidation of adrenaline by ferritin. *J Biol Chem* **220**: 237–255, 1956.
9. Utley HG, Bernheim F and Hochstein P, Effect of sulfhydryl reagents on peroxidation in microsomes. *Arch Biochem Biophys* **118**: 29–32, 1967.
10. Kakkar P, Das B and Vishwanathan PN, A modified spectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophys* **21**: 130–132, 1984.
11. Sinha AK, Colorimetric assay of catalase. *Anal Biochem* **47**: 389–394, 1972.
12. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
13. Summerfield M and Tudhope GR, Studies with primaquine *in vitro*: superoxide radical formation and oxidation of haemoglobin. *Br J Clin Pharmacol* **6**: 319–323, 1978.