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Primaquine Alters Antioxidant Enzyme Profiles in Rat Liver and Kidney

TAPIWANASHE MAGWERE, YOGESHKUMAR S. NAIK and JULIA A. HASLER*

Department of Biochemistry, University of Zimbabwe, Box MP 167, Mt. Pleasant, Harare, Zimbabwe

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The effects of primaquine treatment on antioxidant enzyme activities were investigated in rat liver and kidney. Male Sprague-Dawley rats were treated with 0.21 mg/kg daily for two weeks (chronic treatment) or a single dose at 0.21 or 0.63 mg/kg. Antioxidant enzyme activities were determined in liver and kidney cytosolic fractions whereas glutathione (GSH) and malondialdehyde (MDA) levels were determined in tissue samples. Results for the liver showed increases in cytosolic superoxide dismutase (SOD) and glutathione peroxidase (GPX) enzymatic activities after chronic primaquine treatment. Levels of MDA, a marker for lipid peroxidation, were also increased by more than 50% indicating enhanced oxidative damage in the liver. In the single dose study, 0.63 mg/kg primaquine caused a more than 100% increase in liver SOD and a 36% increase in NAD (P) H: quinone oxidoreductase (NQOR) activities. Results for the kidney, however, showed fewer primaquine-induced changes in antioxidant enzyme activities when compared to the liver in both the chronic and single dose studies. Overall, our results indicate that primaquine treatment causes an oxidative stress in the two rat organs. These results are consistent with the known pro-oxidant effects of primaquine *in vivo*, and supplement current knowledge on the effects of antimalarial drugs on various enzyme systems.

Keywords: Primaquine, antioxidant enzymes, oxidative stress, liver, kidney, rat

Abbreviations: **CAT**: catalase; **GPX**: glutathione peroxidase (selenium dependent enzyme); **GSH**: glutathione (reduced); **MDA**: malondialdehyde; **NQOR**: NAD(P)H: quinone oxidoreductase (DT-diaphorase); **SOD**: superoxide dismutase (cytosolic Cu, Zn form)

INTRODUCTION

Quinoline antimalarial drugs have played a crucial role in the fight against malaria for decades. The emergence of drug tolerance in malaria parasites[1,2] however, has called for further research on antimalarial drugs. Primaquine, a synthetic 8-aminoquinoline, is one of the few quinoline antimalarial drugs with tissue schizontocidal action against relapsing forms of malaria.[3–5] Primaquine, unfortunately, has limited use because of its toxicity in individuals with genetic glucose 6-phosphate dehydrogenase deficiency.[3,6]

Although much is known about the toxicity of primaquine, there is little information on how it affects antioxidant enzymes which protect organs from oxidative damage. A number of studies

* Corresponding author.

have shown the pro-oxidant effects of primaquine in the blood.[7–11] In addition, primaquine metabolites have been shown to undergo redox-cycling both *in vitro* and *in vivo*.[8,12] We have shown previously[13] that chloroquine, a 4-aminoquinoline antimalarial, changes the profile of antioxidant enzyme activities in livers of rats although chloroquine and its metabolits are not known to undergo redox cycling. We postulated that the observed changes in antioxidant enzyme activities might render the organ susceptible to oxidative damage after chloroquine treatment.[13]

Studies on the effect of primaquine on other enzyme systems have shown that primaquine has inhibitory effects on cytochrome P-450s,[14–16] leading to alterations in pharmacokinetics and metabolism of other drugs.[17,18] In the present study we have focused on determining the effects of primaquine on the antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and NAD(P)H (quinone) oxidoreductase (NQOR) in rat livers and kidneys with the aim of adding to current knowledge on effects of primaquine on antioxidant status during treatment with the drug.

MATERIALS AND METHODS

Chemicals

Biochemicals used in the study, including primaquine diphosphate, were obtained from Sigma Chemical Company (MO, USA). All other chemical reagents were obtained from McDonald Scientific (Harare, Zimbabwe) and were of analytical grade.

Animals

Rats were obtained from the University of Zimbabwe Animal House and kept in well ventilated cages (90 cm × 60 cm) with a 12 hours day/night cycle prior to, and during the study period. All animals had access to food and water *ad libitum*, which consisted of Mouse Comproids (National Foods, Ltd., Zimbabwe) and clean tap water. Experiments were carried out when rats were 8 weeks old and weighing between 180–200 g.

Chronic Primaquine Treatment

Twelve male Sprague-Dawley rats were divided into two groups of five and seven animals. To each of the animals in one group (seven animals) was administered 0.21 mg/kg primaquine (0.38 mg primaquine diphosphate/kg in 0.85% saline) by gavage. Animals in the other group of five (controls) were each given a volume of saline equivalent to the primaquine dose. Administration of the drug continued daily (between 8:30 and 9:30 am) for 14 days after which the animals were sacrificed as described later.

Effects of Single Primaquine Doses

In a separate experiment, 15 male Sprague-Dawley rats were divided into three groups of five. In the two treatment groups, rats in one group were each given primaquine (0.21 mg/kg) by gavage as a single dose. Rats in the other group received a single dose of 0.63 mg/kg (1.1 mg primaquine diphosphate/kg) each. The third group of five rats (controls) were given only saline as described earlier. The animals were kept in their respective groups in separate cages for 24 hours until sacrifice.

Preparation of Cytosolic Fractions

Animals were sacrificed by cervical dislocation 24 hours after completion of each dose regimen. Livers and kidneys were perfused *in situ* with ice-cold 0.85% saline through the hepatic portal vein until they had blanched. Small (100—200 mg) pieces from each of the organs were isolated and stored at –80°C to be used for the determinations of glutathione (GSH) and malondialdehyde (MDA) levels. The remaining portions of livers

and kidneys were homogenised in 3 volumes of ice-cold 50 mM potassium phosphate buffer (pH 7.4). Cytosolic fractions were prepared from the homogenates by differential centrifugation.

Assays

Protein determinations in liver and kidney cytosolic fractions were carried out according to Lowry *et al.*[19] The cytosolic SOD activity was measured according to the adrenochrome assay of Misra.[20] CAT and GPX activities were both measured using hydrogen peroxide as substrate according to the methods described by Claiborne[21] and Günzler & Flohé,[22] respectively. NQOR activity was determined using 2,6-dichlorophenolindophenol as substrate.[23] GSH was measured by the fluorometric procedure of Cohn and Lyle[24] and MDA, a product of lipid peroxidation, according to Draper and Hadley.[25]

Absorbances for antioxidant enzyme, protein and MDA assays were measured on a Shimadzu UV-1601 spectrophotometer (Shimadzu Corporation). GSH measurements were performed using a model 1000 Perkin-Elmer fluorescence spectrophotometer.

Statistical Analyses

Numerical results were expressed as mean ± the standard deviation (SD). Differences between controls and treated animals were tested for statistical significance by the unpaired Student *t*-test.

RESULTS

Effects of Chronic Primaquine Treatment

Table I shows the effects of chronic primaquine administration on antioxidant enzyme activities, GSH and MDA levels in rat livers and kidneys. Increased cytosolic Cu, Zn-SOD, GPX and MDA were observed in the livers of primaquine-treated rats following administration of the drug, with no significant alterations in any of the other parameters. In kidneys, only GPX and MDA levels were significantly increased and decreased, respectively, in treated animals.

Effects of Single Primaquine Doses

From Table II, it will be observed that single doses of primaquine produced fewer changes in antioxidant enzyme parameters in rat livers and kidneys when compared to the chronic dose (Table I). Both doses increased NQOR activity in livers of treated animals, whereas only the higher dose significantly increased SOD activity in the same organ. In the kidneys, the higher dose of primaquine decreased only NQOR activity, an effect which is opposite to that observed for the liver.

DISCUSSION

The treatment for relapsing malaria is 15 mg primaquine (0.21 mg base/kg for a 70 kg adult) daily for 14 days.[3] A single dose of 45 mg (0.63 mg base/kg) has been advocated in order to prevent spread of drug resistance.[3,4] The primaquine doses given to rats in this study, therefore, simulate dose regimens for malaria treatment in man. The liver is the site of activity of primaquine as a tissue schizontocide as well as its metabolism in several animal species.[4,26]

Taken orally, primaquine is completely absorbed from the gut and its elimination half-life ranges from 5–6 hours in humans.[3,4] Two primaquine metabolites, carboxyprimaquine and 6-methoxy-8-aminoquinoline have been characterised in man.[26] In rats, primaquine is metabolised to 5, 6-dihydroxy-8-aminoquinoline when administered orally.[27] The other metabolites of primaquine identified in rats include the urinary metabolite 5-hydroxy-demethylprimaquine, and a carboxylic acid derivative in plasma.[26,27] These metabolites are among those

TABLE I The effects of chronic treatment with primaquine (0.21 mg/kg daily for 14 days) on antioxidant enzyme activities, reduced glutathione and malondialdehyde levels in rat livers and kidneys[a]

	LIVERS		KIDNEYS	
	Controls (n = 5)	Treated (n = 7)	Controls (n = 5)	Treated (n = 7)
SOD	293 ± 32	374* ± 63	264 ± 47	286 ± 170
CAT	781 ± 97	782 ± 87	282 ± 29	329 ± 170
GPX	0.75 ± 0.05	1.08* ± 0.26	0.51 0.02	0.64** ± 0.05
NQOR	79 ± 29	85 ± 33	19 ± 5	20 ± 4
GSH	3.63 ± 0.51	3.36 ± 0.34	1.41 ± 0.08	1.37 ± 0.16
MDA	33 ± 6	51* ± 8	37 ± 8	23* ± 7

[a] CAT, SOD and GPX activities are expressed as units/mg protein, whereas GSH and MDA levels are μmol/g of tissue. NQOR activity is in μmol 2,6-dichlorophenolindophenol reduced/min/mg protein. All results are expressed as mean ± SD for the number of animals indicated in parentheses. Values significantly different from controls by Student's *t*-test are: *$p < 0.05$ and **$p < 0.02$.

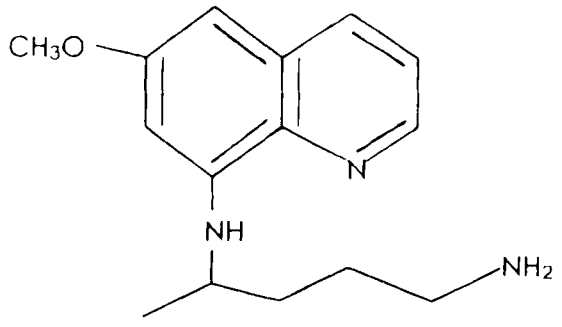
that have been demonstrated to cause methaemoglobin formation both *in vitro*,[8,28] and *in vivo*,[8,27] indicating oxidative damage to erythrocytes. Figure 1 shows the structures of primaquine and its human and rat metabolites.

In the chronic primaquine study (Table I), results for the liver show that primaquine treatment leads to increased SOD, GPX and MDA levels (130%, 144% and 155% respectively with respect to controls). The two enzymes, SOD and

TABLE II Dose dependent effects of primaquine on antioxidant enzymes, glutathione and malondialdehyde levels in rat livers and kidneys[b]

	LIVER			KIDNEY		
	Control	0.21 mg/kg	0.63 mg/kg	Control	0.21 mg/kg	0.63 mg/kg
SOD	280 ± 22	344 ± 92	568* ± 234	225 ± 42	249 ± 54	258 ± 45
CAT	898 ± 66	910 ± 108	956 ± 84	269 ± 90	209 ± 60	483 ± 111
GPX	0.68 ± 0.10	0.59 ± 0.05	0.57 ± 0.03	0.43 ± 0.07	0.40 ± 0.07	0.34 ± 0.05
NQOR	88 ± 14	118** ± 16	120** ± 8	21 ± 4	18 ± 2	15* ± 3
GSH	2.10 ± 0.42	2.49 ± 0.36	2.58 ± 0.21	1.47 ± 0.04	1.91 ± 0.22	1.62 ± 0.25
MDA	38 ± 6	55 ± 10	41 ± 16	57 ± 34	44 ± 42	24* ± 11

[b] CAT, SOD and GPX activities are expressed as units/mg protein, whereas GSH and MDA levels are μmol/g of tissue. NQOR activity is μmol 2,6-dichlorophenol-indophenol reduced/min/mg protein. Each value represents Mean ± SD for five animals per group and all abbreviations are as given in the text. Values significantly different from controls are: *$p < 0.05$ and **$p < 0.01$.



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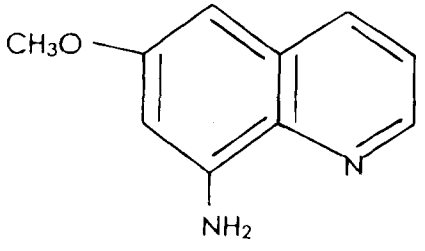

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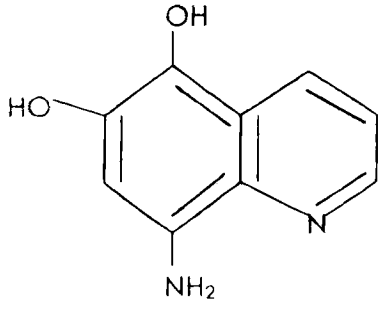

III

FIGURE 1 The chemical structures of primaquine and some of its metabolites: **I**, primaquine; **II**, 6-methoxy-8-aminoquinoline (in human); **III**, 5,6-dihydroxy-8-aminoquinoline (in rats).

GPX, are known to be induced by reactive oxygen species (ROS) such as the superoxide anion radical[29,30,31] and hydrogen peroxide,[29] in bacteria. Mechanisms of induction of these two enzymes are not as straight-forward in mammalian species as in bacteria. We can, however, postulate that the increased SOD and GPX activities in our study reflect a response of the liver to the oxidative stress due to redox cycling of primaquine metabolites *in vivo*.[8] Further investigations will be required, however, to determine specific mechanisms for the effects of primaquine on rat liver SOD. It appears from our results that primaquine treatment does not cause oxidative stress in the kidney, as indicated by lower MDA levels following treatment. Effects of primaquine were greater in liver compared to kidney possibly because most of primaquine metabolism occurs in the liver.

In comparing the chronic effects of the 8-aminoquinoline (primaquine) with the 4-aminoquinoline (chloroquine),[13] we note that SOD levels are increased by both drugs. The two drugs, however, have differing effects on GPX, CAT and NQOR. Chronic chloroquine treatment lowered the activities of all these enzymes in the liver whilst chronic primaquine treatment raised GPX activity and had no effect on CAT and NQOR. The differences between primaquine and chloroquine may arise as a result of the redox-cycling of primaquine metabolites;[8,12] no reports of redox-cycling have been made for chloroquine or its metabolites.

In the single dose study (Table II), rat livers showed a different pattern of alterations in antioxidant enzyme activities compared to the chronic dose study (Table I). For both primaquine and chloroquine,[13] it is clear that single dose and chronic dose effects differ qualitatively and quantitatively and this reflects the differing adaptive responses to single and chronic stimuli. For the single dose study, SOD activity was increased by primaquine at 0.63 mg/kg (200% versus 130% in chronic study). This increase in SOD activity was observed 24 hours after the pri-

maquine treatment and indicates a fairly rapid induction of the enzyme in rat liver. Our results are consistent with those of other workers who observed increases in SOD activity of between 15–35% only 4 hours after X-irradiation in spleen and liver of rats.[32] These rapid responses to an oxidative stress underscore the crucial role played by SOD in the detoxification of ROS in organs. It should be noted that the cytosolic Cu, Zn-SOD was investigated here, and that this study did not address the effects of primaquine on the mitochondrial Mn-SOD, although it is very likely that primaquine also affects levels of this enzyme.

In the single dose study, NQOR activity was increased significantly (135%) by both the low and the high doses of primaquine in contrast to a lack of effect in the chronic dose study. This enzyme is involved in a one step two-electron reduction of quinoid compounds, thus preventing their redox-cycling.[23] The increased NQOR activity observed in the liver may be due to primaquine metabolites that are capable of undergoing redox-cycling. Further studies are needed to verify whether such quinone intermediates are capable of inducing NQOR activity and to determine the reasons for a return to control levels of the enzyme after chronic primaquine treatment. In the single dose study with chloroquine,[13] NQOR activity was also increased (in contrast to a decrease seen with chronic chloroquine treatment) but one cannot postulate redox-cycling of metabolites as the reason in this case. It is also possible, therefore, that 4- and 8-aminoquinolines have other effects in common that lead to increases in NQOR after a single dose.

The results reported here indicate that primaquine treatment in rats imposes an oxidative stress on livers and kidneys as shown by the increases in certain antioxidant enzyme activities. A similar conclusion was reached for chloroquine treatment[13] although the specific changes in antioxidant enzymes are different for the two drugs. It now follows that the physiological and clinical implications of these results for primaquine and for chloroquine treatment[13] need to be investigated.

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