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EFFECTS OF CHLOROQUINE TREATMENT ON ANTIOXIDANT ENZYMES IN RAT LIVER AND KIDNEY

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Abstract—The effect of chloroquine (CHQ) administration on antioxidant enzymes in rat liver and kidney was studied. Male Sprague-Dawley rats were administered 20 mg/kg CHQ once a week for 4 weeks (chronic treatment) or a single dose at 10 or 20 mg/kg (acute treatment). Antioxidant enzyme activities were determined in cytosolic fractions of liver and kidney, whereas reduced glutathione (GSH) and malondialdehyde (MDA) were determined in tissue samples. Results indicate minimal effects of acute CHQ treatment, whereas chronic treatment with CHQ differentially affected antioxidant enzymes in the two organs. Superoxide dismutase activity was increased nearly twofold, while activities of selenium glutathione peroxidase (GPX), catalase, and NAD (P) H: quinone oxidoreductase were decreased in livers of CHQ-treated rats compared to controls. No significant effects of CHQ on glutathione reductase, GSH, and MDA levels were seen in the liver. Fewer effects of CHQ were observed in the kidney where a decrease in GPX activity and an increase in MDA levels was seen. Lowering of antioxidant enzymes activities in the liver by CHQ could render the organ more susceptible to subsequent oxidative stress; while increased MDA production after CHQ treatment in the kidney indicate that the organ is being subjected to oxidative stress. This could have implications for prolonged chloroquine intake. Copyright © 1996 Elsevier Science Inc.

Keywords—Antioxidant enzymes, Chloroquine, Oxidative stress, Liver, Kidney, Rats, Free radicals

INTRODUCTION

Chloroquine (CHQ) is a 4-aminoquinoline drug (Fig. 1) that is used widely for both treatment and prophylaxis of malaria.1 Despite widespread resistance to the drug that has evolved in parasites worldwide,2,3 CHQ has remained the mainstay of therapeutic and prophylactic regimens available in most malaria-endemic Third World countries because it is cheaper and more readily available than other antimalarials.

It has been shown that CHQ accumulates in various organs of the body and is eliminated slowly.4 Earlier studies have indicated that antimalarials such as CHQ and primaquine inhibit cytochrome P-450–mediated mixed function oxidase activities both in vivo and in vitro,2,3 but little is known about their interactions with antioxidant enzymes. One study has shown that CHQ causes increased lipid peroxidation and decreased antioxidant enzyme activities in the retina of rat.9 It has also been shown that CHQ and other 4-aminoquinolines adversely affect lysosomal function,10–12 and CHQ has been shown to alter kidney structure and affect renal sodium excretion.13,14 It is possible that CHQ, acting directly or indirectly, could alter antioxidant status and make certain organs more susceptible to the effects of oxidative stress.

Because CHQ is among the most commonly ingested drugs in malaria endemic areas, there is a need to study its effects on antioxidant enzymes in organs where the drug accumulates so as to gain insight into the effect of the drug on antioxidant status during long-term CHQ intake. We report here on the effects of chronic CHQ administration on activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), NAD (P) H: quinone oxidoreductase (QOR), glutathione reductase (GRD), and levels of reduced glutathione (GSH) and malondialdehyde (MDA) in rat liver and kidney.
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MATERIALS AND METHODS

Chemicals

Chloroquine diphosphate was obtained from CAPS Holdings (Ltd) Zimbabwe. All other chemicals and biochemicals were obtained from Sigma Chemical Company (St. Louis, MO), and were of analytical grade.

Treatment of animals

Six-week-old male Sprague–Dawley rats bred in the University of Zimbabwe animal house were allowed access to food and water ad lib and were subjected to 12-h day/night cycles prior to, and during the study period.

Effects of chronic CHQ treatment. Rats were divided into two groups of five each. One group was given CHQ by gavage as CHQ diphosphate in 0.9% saline (10 mg/ml) at a dose of 20 mg/kg body weight. Rats in the second group acted as controls and were each given a volume of saline equivalent to the volume of CHQ. The drug was administered once a week (between 0900 and 0930 h) for 4 weeks. All animals were sacrificed 72 h after the last dose was administered.

Effects of acute CHQ treatment. Fifteen rats were divided into three groups of five. Two of the three groups were given, respectively, 10 and 20 mg/kg CHQ diphosphate in 0.9% saline as a single dose by gavage. Animals in the third group, the control group, were only given saline. Animals were sacrificed 72 h after CHQ treatment.

Preparation of cytosolic fractions

Rats were sacrificed by cervical dislocation and whole animals were perfused with 0.9% saline through the left ventricle of the heart until the livers and kidneys had blanched. Small pieces of the individual livers and kidneys (0.1–0.2 g each) were excised and immediately frozen at −80°C for the determinations of GSH and MDA.

The remaining portions of the livers and kidneys were minced with scissors in 3 volumes of ice-cold 100 mM potassium phosphate buffer, pH 7.4, and homogenized in a Potter-Elvehjem homogenizer. Homogenates were first centrifuged at 10,000 × g for 15 min and the supernatants were then centrifuged at 100,000 × g for 1 h. All operations were carried out at 4°C. The resulting supernatants, termed cytosolic fractions, were aliquoted and stored at −80°C until required for analysis of enzyme activities.

Assays

Protein determinations in liver and kidney cytosolic fractions were carried out according to the method of Lowry et al. using crystalline bovine serum albumin as standard. SOD activity was measured according to the method based on inhibition of autoxidation of epinephrine at pH 10.2 at 30°C. CAT activity was assayed by monitoring the decomposition of hydrogen peroxide at 240 nm. GPX activity was measured by the coupled assay using hydrogen peroxide as substrate, azide (1 mM) was included in the assay mixture to inhibit CAT. QOR activity was determined using either menadione (MEN) or 2,6-dichlorophenolindophenol (2,6-DCPIP) as substrate. GRD activity was assayed by the method described by Carlberg and Mannervik. GSH was measured by the fluorimetric procedure of Cohn and Lyle and MDA, a product of lipid peroxidation, as described by Draper and Hadley.

Effects of CHQ on antioxidant enzymes in vitro

Cytosolic fractions from saline-treated animals (controls) were used in these assays. CHQ diphosphate was added to each of the assay mixtures for SOD, CAT, GPX, and QOR at a final concentration of 0, 10, 25, 50, 75, and 100 µM and the assays conducted as described above.

Statistical analysis

Tests for statistical significance between controls and CHQ-treated animals were done using the unpaired Student’s t-test.

RESULTS

Table 1 shows the results of chronic CHQ treatment (four doses over 4 weeks) on cytosolic antioxidant enzymes, GSH, and MDA levels of rat liver and kidney.
Table 1. Effect of Chronic Chloroquine Treatment on Antioxidant Enzymes, Reduced Glutathione, and Malondialdehyde Levels in the Rat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Liver</th>
<th></th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD((a)) (Units/mg)</td>
<td>Controls 47.1 ± 4.1</td>
<td>Treated 88.9 ± 5.9</td>
<td>% Control 187</td>
</tr>
<tr>
<td>GPX((a)) (Units/mg)</td>
<td>Controls 1.82 ± 0.11</td>
<td>Treated 1.07 ± 0.12</td>
<td>59</td>
</tr>
<tr>
<td>CAT((a)) (Units/mg)</td>
<td>Controls 25.6 ± 1.8</td>
<td>Treated 19.9 ± 1.5</td>
<td>78</td>
</tr>
<tr>
<td>QOR MEN((a))</td>
<td>Controls 191.1 ± 22.7</td>
<td>Treated 93.4 ± 22.5</td>
<td>49</td>
</tr>
<tr>
<td>2,6-DCPIP</td>
<td>Controls 110.3 ± 12.0</td>
<td>Treated 39.9 ± 7.8</td>
<td>36</td>
</tr>
<tr>
<td>GRD((a))</td>
<td>Controls 60.3 ± 10.5</td>
<td>Treated 52.7 ± 3.7</td>
<td>87</td>
</tr>
<tr>
<td>GSH (µmol/g)</td>
<td>Controls 2.05 ± 0.25</td>
<td>Treated 1.88 ± 0.38</td>
<td>92</td>
</tr>
<tr>
<td>MDA (µmol/g)</td>
<td>Controls 27.4 ± 2.8</td>
<td>Treated 25.4 ± 3.6</td>
<td>93</td>
</tr>
</tbody>
</table>

\(a\) All enzyme activities are expressed per mg protein whereas GSH and MDA are per gram of wet tissue.

1 Unit of SOD is the amount of enzyme that inhibits autoxidation of epinephrine by 50% at pH 10.2, 30°C.

1 Unit of GPX represents pmoles NADPH oxidised/min at pH 7.0 and 37°C.

1 Unit of CAT decomposes 1 µmol H2O2/min at pH 7.0 and 25°C.

QOR activities are pmol cytochrome c or 2,6-DCPIP reduced/min at pH 7.5 and 30°C with NADH as electron donor.

GRD activity is in µmol NADPH oxidized/min. See text for abbreviations.

NS = Not significant at p < .05. All other abbreviations are as described in the text.

Body, liver, and kidney weights were also measured and no statistically significant differences were observed between the controls and CHQ-treated groups (data not shown). In the livers of CHQ-treated rats, there was an almost twofold increase in the activity of SOD compared to controls, while GPX, CAT and QOR activities were reduced significantly. Of the latter enzymes, QOR activity was decreased to the greatest extent by a factor of 2.0 and 2.8 with MEN and 2,6-DCPIP, respectively. GRD activity was not significantly altered by chronic CHQ administration in the liver. The cellular redox status of the liver, as indicated by levels of GSH, was not affected by chronic CHQ because there was no significant change in this thiol. In addition, MDA levels, which were used as an index of lipid peroxidation, were not altered due to chronic CHQ treatment.

In kidneys, fewer effects were seen and the degree of statistical significance for the changes were less. The only significant change in enzyme levels as a result of chronic CHQ treatment in the kidney were for GPX (p < .0001). MDA levels, although not altered by CHQ in the liver, were elevated (p < .009) in the kidneys of CHQ-treated rats when compared to controls.

In order to determine whether the effects of CHQ on antioxidant enzymes would be seen after an acute exposure, two groups of rats were treated with a single dose of CHQ each (10 and 20 mg/kg). Levels of antioxidant enzymes, GSH, and MDA 72 h after treatment are shown in Figs. 2 and 3. In contrast to the effects seen after chronic CHQ treatment, there were no observed effects of acute CHQ treatment in either organ except for a 1.4-fold increase (p < .05) in the activity of QOR produced by treatment with 10 mg/kg CHQ in liver. The results also show that neither dose of CHQ altered the GSH levels in either liver or kidney. The higher dose of CHQ (20 mg/kg) led to a significant decrease (p < .01) in MDA levels in the liver and an increase (p < .05) in the kidney. The kidney appears to be subject to oxidative stress on the basis of MDA production after either acute or chronic exposure to CHQ.

There is the possibility that the observed effects of CHQ on antioxidant enzymes in the chronic study were an artefact of the drug present in the cytosolic fractions. This is, however, unlikely as evidenced by the results of the acute CHQ study where the drug would also be present, yet no changes in enzyme activities were seen. It was still of interest, however, to determine the effect of CHQ on antioxidant enzymes in vitro by adding CHQ to the assay mixtures. The results (Fig. 4) show that CHQ inhibits antioxidant enzyme activity to only a limited extent. The highest concentration of CHQ used was 100 µM. Even at this concentration, inhibition of enzyme activity was generally less than 30%, the exception being GPX in the kidney, where about 40% inhibition was obtained. In no case was it possible to calculate an I₅₀ value for CHQ inhibition of the enzymes.

DISCUSSION

One observation of this study is that the effects of CHQ differ between the rat liver and kidney. The explanation for this could lie in differences in regulation of expression of antioxidant enzymes in the two organs as many tissues show separate and unique responses to factors that regulate antioxidant enzymes. It is possible that the two organs accumulate CHQ to different extents as was observed by other workers. As such, cells in different organs may not be exposed to the same levels of CHQ and are affected differently. Bhattacha-
ryya et al. found no effect of CHQ on SOD of rat retina at doses between 1 and 10 mg/kg given over 7 or 15 d. They did observe a slight increase in SOD in the first few hours after administration of the drug at 5 and 10 mg/kg. Our results for GPX and CAT in the liver are similar to those seen in rat retina where a decrease was observed in these enzymes after both acute and daily chloroquine treatment over 15 d.
Chloroquine and antioxidant enzymes

A therapeutic dose of CHQ was shown to lead to a decrease in protein turnover in humans, and treatment of rats with CHQ over 3 d decreased the levels of hepatic cytochrome P450. In our study, we report an increase in SOD activity and decreases in GPX, CAT, and QOR in the liver after chronic, but not acute, CHQ treatment. Our observations need to be followed up with studies to determine the level (transcriptional, translational, protein stabilization, etc.) at which expression of these enzymes is affected by chloroquine. Factors regulating the expression of antioxidant enzymes have been studied in many laboratories with much of the work having been done in prokaryotic systems. In higher animals, influence of factors such as organ specific expression, stage of development of the organism, and hormonal status are likely to affect regulation of antioxidant enzymes.

There are three forms of SOD found in prokaryotes and eukaryotes characterized by their different metal ion cofactors. It was the cytosolic copper, zinc-SOD that was the subject of this study. Despite the wide distribution of SOD in both mammalian and non-mammalian species, the regulation of this enzyme in mammals is still not clear. In prokaryotes, several factors such as hyperoxia, changes in metabolic rate, nutritional status, and increased intracellular and extracellular fluxes of the superoxide anion are known to influence biosynthesis of SOD. The control of SOD synthesis in response to superoxide generators such as quinones, violagens, and some redox cyclers, such as paraquat and diquat, has also been reviewed. Although some metabolites of primaquine, an 8-amino-quinoline antimalarial, have been shown to be redox cyclers, there are no reports to indicate that chloro-
quine or any of its metabolites behave in this way. The increased SOD levels in the livers of chronic CHQ-treated rats observed here could, therefore, be due to the organ’s response to an increased production of reactive oxygen species, as a result of exposure to CHQ or its metabolites.

The finding of decreased GPX and CAT activities in the liver as a result of chloroquine treatment is somewhat surprising in view of the increased SOD levels. One might have expected induction of GPX and CAT if the increased SOD levels led to an increased production of hydrogen peroxide within the liver and/or if chronic CHQ treatment is leading to an oxidative stress. Oxidative stress in the form of endurance training has been shown to simultaneously increase CAT, GPX, and GRD in heart and skeletal muscle. Another example where coregulation of these enzymes appears to occur is in response to some cytokines and hormones. Rats exposed to ozone for a short time develop increased levels of CuZnSOD, MnSOD, CAT, and GPX in lung tissue and a tolerance to ozone. Harris highlights, however, that attempts to understand antioxidant enzyme regulation have focused on single enzymes responding to specific oxidant signals. Thus, it is difficult to compare the results of our study with those in the literature. The observation that GPX and CAT were both lowered by CHQ in the liver, however, suggests that this organ may be susceptible to hydrogen peroxide-induced oxidative stress after CHQ treatment.

QOR from hepatic and other cells has been reported to be inducible by a large number of xenobiotics and to be upregulated in the livers of hepatocarcinoma patients. In contrast, in the present study, chronic CHQ treatment decreased QOR activity by almost 50% in the liver with both MEN and 2,6-DCPIP as substrates. Whether this effect of CHQ is due to a general effect of CHQ on protein turnover or a more direct effect on gene expression requires further study.

The level of reduced glutathione is a measure of the cellular redox status. Our results show that chronic CHQ treatment appears not to affect the overall redox status of the cell in both liver and kidney as indicated by a lack of alteration in the levels of the thiol in both organs. In contrast, other workers found that GSH levels in rat retina were decreased up to 4 h after a single dose of CHQ, but increased after 7 or 15 d. The lack of any change in GSH levels in liver and kidney after 4 weeks of CHQ administration in our study could be due to organ-specific differences in the effect of the drug on enzymes involved in GSH metabolism.

The suitability of the thiobarbituric acid (TBA) assay as a reliable means of monitoring lipid peroxidation in crude samples by measuring MDA levels has been debated and some doubts on its reliability expressed. The TBA assay has, however, been shown to be a reliable and satisfactory means of assessing lipid peroxidation in most biological samples. Based on our MDA results alone, it appears chronic chloroquine treatment caused increased lipid peroxidation in the kidney. It is possible, given the results of our in vitro inhibition studies, that CHQ is inhibiting GPX in the kidney. It would appear that the kidney is less able to protect itself from the effects of the drug than is the liver, and this could have implications for renal function in individuals on long-term CHQ prophylaxis.

In conclusion, we have shown that the profile of antioxidant enzymes is altered in the liver, but not in the kidney, as a result of chronic CHQ treatment. The mechanism for the alterations have yet to be investigated, as do the significance of these changes for the ability of the liver to protect itself from oxidative stress.

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REFERENCES


ABBREVIATIONS

2,6-DCPIP — 2,6-dichlorophenolindophenol
CAT — catalase
CHQ — chloroquine
GPX — selenium containing glutathione peroxidase
GRD — glutathione reductase
GSH — reduced glutathione
MDA — malondialdehyde
QOR — NAD (P) H:quinone oxidoreductase
SOD — superoxide dismutase