Incidence of Chloroquine Induced Oxidative Stress in the Blood of Rabbit

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Abstract: The effect of single dose chloroquine treatment on enzymatic antioxidant profiles and lipid peroxidation in the blood of rabbits was investigated. Treatment of rabbits with 10 mg kg⁻¹ (single dose) chloroquine resulted in increases of 53.6, 11.6 and 3.6% (p<0.05) in the activity of superoxide dismutase at 6, 12 and 24 h, respectively after drug administration while the activity of catalase was significantly reduced by 37.1, 15.5 and 2.9% (p<0.05) at the same intervals when compared with control. Malondialdehyde level increased (p<0.05) by 11.9 and 1.9% at 6 and 12 h, respectively, but decreased by 13.8% at 24 h relative to control. Reduced glutathione was decreased by 41.9, 38.7 and 3.2% at the same hourly intervals when compared with control (p<0.05). The findings in this study suggest that chloroquine treatment altered enzymatic antioxidant defense systems and elevated lipid peroxidation in the blood of rabbits with the most noticeable alteration observed 6 h after drug administration perhaps due to the peak plasma concentration of chloroquine which is usually reached between 3 and 5 h after drug administration. Oxidative stress is thus induced in the blood by chloroquine at least within 6 h of its administration.

Key words: Chloroquine, oxidative stress, antioxidant status, lipid peroxidation

INTRODUCTION

Chloroquine, a 4-aminoquinoline is a rapidly acting blood schizontocide, which is normally effective against the erythrocytic stage of all the four plasmodia species, which cause human malaria[9]. A chlorine atom attached to position 7 of the quinoline ring is believed to confer the greatest antimalarial activity in both avian and human malarial species.

Chloroquine, despite the emergence and spread of resistance to it by malaria parasites still remains a first line drug in some endemic areas. It is also the reference drug in the evaluation of malaria chemotherapy with other agents. Doses of chloroquine used for the treatment of acute malarial attack may cause some adverse events such as gastro-intestinal upset, transient mild headache and visual disturbances. When taken in proper doses, it is however an extraordinarily safe drug. However, high daily doses (>250 mg) used for treatment of diseases other than malaria can result in irreversible ototoxicity and retinopathy[10]. Many of the known biological effects of chloroquine are thought to be directly related to its lysosomotropism[11,12]. The mechanism of chloroquine resistance remains to be clearly defined. Fong et al.[9] proposed free radical pathology in lysosomal destabilization. However, it is being widely accepted that chloroquine by virtue of its weak base properties accumulates in the vacuole where it exerts antimalarial properties by inhibiting the process of heme polymerization and detoxification which eventually results in the death of the parasites[6,7,8].

There is considerably high evidence to show that the host red blood cells are exposed to oxidative stress during malaria[10] and that decreased protection against oxidative damage may inhibit parasite growth in erythrocytes with glucose-6-phosphate dehydrogenase (G6PD) deficiency. A number of radical generating antimalarias such as primaquine appear to operate on the principle that oxidative damage has a more deleterious effect on the parasite than on the host cells. However, due consideration is not accorded to the long-term effect and spontaneity of the radical chain reaction on cellular tissues of host cells. Pathological conditions set in when the free radicals generated overwhelm the body’s natural antioxidant defense systems. Free radicals propagate chain reactions that attack cellular DNA, proteins, lipids
and carbohydrates thereby causing degenerative diseases such as cancer, inflammation, apoptosis, ageing and diabetes. \cite{30,31}

Cellular antioxidants, which include scavenger enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH px) and catalase (CAT) as well as fat-soluble vitamins (vitamins A and E) and water-soluble low molecular weight substances (vitamin C, glutathione and carotenoids), function to detoxify free radicals \cite{11}. SOD specifically promotes the dismutation of superoxide anion \((O_2^-)\) into \(H_2O_2\), which can be detoxified by both CAT and GSH px. \cite{10}

Chloroquine against all odds has remained the mainstay of therapeutic and prophylactic regimen available in most malaria endemic countries primarily because it is relatively cheap and readily available than the alternative antimalarial drugs. It is also usually the drug of preference in the evaluation of chemotherapy of malaria with other agents. There is however, little knowledge presently on its effect on cellular antioxidant defense systems and extent of lipid peroxidation, especially in the blood, during its use in malaria therapy.

In view of this, the present study was undertaken to determine the extent of chloroquine induced-oxidative stress on cellular enzymatic antioxidant defense system in the blood of rabbits.

**MATERIALS AND METHODS**

**Chemicals:** Chloroquine (Chloroquine Phosphate BP of Evans Pharmaceutical Company, Lagos) was obtained from the University College Hospital, Ibadan, Nigeria. Glutathione, Thiobarbituric acid, Epinephrine, 5,5-dithio bis (2-Nitrobenzoic acid) and hydrogen peroxide were purchased from Sigma chemical Co., London, UK. All other reagents were of analytical grade and were obtained from the British Drug House, Poole, UK.

**Animal and treatment:** Ten male rabbits weighing between 720 and 800 g were obtained from the animal house at the department of Biochemistry, University of Ibadan, Nigeria. They were kept in wire-meshed cages and fed with standard Guinea feed pellet (Ladokun feeds, Nigeria Limited) and liberally supplied with water throughout the period of the experiment. The rabbits were divided into 2 groups of 5 animals each. The first group was treated with an oral single dose of chloroquine (10 mg kg\(^{-1}\) body weight) while the second group, which served as controls received physiological saline.

**Blood collection:** Blood samples (5 mL each) were collected from the marginal veins of the outer ear of the rabbits into heparinised tubes and into plain sterile tubes before and at 6, 12 and 24 h after drug administration. Whole blood was used for CAT and SOD assays while the serum was used for protein and lipid peroxidation determination.

**Assays for antioxidant enzymes:** SOD activity was determined in the blood by measuring the inhibition of autoxidation of epinephrine at pH 10.2 and 30°C by the method of Misra and Fridovich \cite{13} as described by Tapiwansahe et al. \cite{14}.

Activity of CAT was determined according to the procedure of Claiborne \cite{15} by following the absorbance of hydrogen peroxide at 240 nm at pH 7.0 and 25°C.

Serum GSH level was determined according to the procedure of Ellman \cite{16}.

Serum lipid peroxidation was assessed by the formation of malondialdehyde as described by Gutteridge and Wilkins \cite{17}.

Protein content of all samples was estimated by the method of Lowry et al. \cite{18} using bovine serum albumin as standard.

**Statistics:** The data were analysed by a two-tailed student’s t-test. \(P < 0.05\) were considered statistically significant between controls and treated rabbits.

**RESULTS**

Results showed that there was a significant increase in the activity of erythrocyte SOD at 6 h (17.2±7.4 \(\mu\)mol mg\(^{-1}\) protein) after drug administration relative to 11.2±0.5 \(\mu\)mol mg\(^{-1}\) protein, while a progressive decrease in activity was observed afterwards as it tended to normal by 24 h (11.6±1.4 \(\mu\)mol mg\(^{-1}\) protein) (Table 1). Furthermore, present data revealed a significant reduction (37%) in the activity of CAT at 6 h (34.4±2.9 \(\mu\)mol H\(_2\)O\(_2\) consumed/min/mg protein) after drug administration relative to control but thereafter increased progressively towards the control value (54.7±1.0 \(\mu\)mol H\(_2\)O\(_2\) consumed/min/mg protein) at 12 and 24 h of chloroquine administration.

MDA level was raised by 9.8% at 6 h (17.9±10.1 mmole MDA formed/mg protein) after drug administration relative to control with the level tending towards the control value at 12 and 24 h after (Table 2). GSH level decreased significantly by 41.9% at 6 h (1.8±0.1 nmol) after drug administration relative to control with the level returning to the control value at 12 and 24 h.
<table>
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<tr>
<th>Table 1: Effect of single dose (10 mg kg⁻¹) chloroquine treatment on antioxidant enzymes in rabbits</th>
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<td>Parameter</td>
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</tr>
<tr>
<td>SOD (µmol mg⁻¹ protein)</td>
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<tr>
<td>CAT (µmol H₂O₂ consumed/min/mg protein)</td>
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The enzyme activities are expressed per mg protein. 1 unit of superoxide dismutase (SOD) is the amount that inhibits autoxidation of epinephrine by 50% at pH 10.2 at 30°C. 1 unit of catalase (CAT) decomposes 1 µmol H₂O₂ /min at pH 7.0 and 25°C. Values are expressed as mean±SD for 5 rabbits in each group. *Significantly different from control (p<0.05)

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<th>Table 2: Effects of single dose (10 mg kg⁻¹) chloroquine treatment on the extent of lipid peroxidation and GSH level in the serum of rabbits</th>
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<td>Parameter</td>
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<td>MDA (umole mg⁻¹ protein)</td>
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Malondialdehyde (MDA) and reduced glutathione (GSH) are expressed per mg protein. The values are the mean±SD for 5 rabbits in each group. *Significantly different from control (0 h) at p<0.05

**DISCUSSION**

The present study demonstrates that chloroquine treatment alters the enzymatic antioxidant profiles in the blood. Single dose treatment resulted in increased SOD activity and MDA values, while a decrease in CAT and GSH activities were observed at 6 h after drug administration. However, the levels of the parameters tended towards the control at 12 and 24 h of chloroquine administration.

Chloroquine is known to be well absorbed from the gastrointestinal tract and rapidly from intra-muscular and subcutaneous sites. The volume of distribution of the drug is very large (100 to 1000 L kg⁻¹) due to its extensive sequestration in tissues, particularly in the liver, spleen, kidney, lung, melanin containing tissues and to a lesser extent, brain and spinal cord. It concentrates in parasitized red cells. The half-life of chloroquine increases from a few days to weeks as plasma level declines, neglecting transition from slow distribution to even slower elimination from extensive tissue stores. It binds moderately (50%) to plasma protein and undergoes appreciable biotransformation with peak plasma levels achieved within 3 to 5 h of drug administration. This perhaps is the reason for the significant modulatory effect of chloroquine as observed at 6 h after drug administration compared with its effect at 12 and 24 h. The drug is known to be eliminated gradually afterwards, thus explaining the pattern of restoration of the altered enzyme activities.

SOD is a metalloenzyme that catalyzes the dismutation of superoxide anion (O₂⁻) into H₂O₂ and O₂·⁻. It may be particularly important for mammalian erythrocytes because activated oxygen species, OH, H₂O₂ and O₂·⁻ forms spontaneously within the cells. This increase of SOD activity is in agreement with previous findings who reported a slight increase in SOD activity of rat retina in the first few hours of chloroquine administration at 5 and 10 mg kg⁻¹, although they found no effect at doses below 10 mg kg⁻¹ over 7 or 15 days of administration. It also agrees with reports of Tapiwanashe et al. and Farombi et al. This suggests that a state of oxidative stress is generated in the blood of rabbit in response to chloroquine administration.

However, the concentrations of GSH and CAT activity reduced significantly (p<0.05) although tended towards the control values at 12 and 24 h of drug administration. This agrees with the findings of Bhattacharyya et al. who reported a similar trend in rat retina and Tapiwanashe et al. in the liver of chloroquine treated rat.

Catalase is a tetrameric haemoprotein that undergoes alternate divalent oxidation and reduction at its active site in the presence of H₂O₂. Studies have shown that it can be inhibited by superoxide anion, which converts it to ferroxyl and ferryl states, the inactive forms of the enzyme. The induction of catalase is expected, if increased SOD level led to an increased production of H₂O₂ in the blood or if chloroquine treatment led to oxidative stress. However, decreased CAT activity by chloroquine observed in the present study could lead to the accumulation of H₂O₂ and aggravate oxidative stress. Catalase is known to remove H₂O₂ when present at high concentration while GSH peroxidase removes H₂O₂ when present at steady-state. The decreased catalase activity reported in this study therefore suggests that the blood may be susceptible to H₂O₂ induced oxidative stress following chloroquine administration. Chloroquine has been shown to be an efficient inhibitor of the catalase activity of heme.

The increased MDA formation in the blood following chloroquine treatment suggests the induction of lipid peroxidation by this drug. Thus, the toxicity of oxidant species is enhanced in the presence of chloroquine. Indeed, chloroquine has been shown to enhance the ability of heme to catalyse lipid peroxidation. The involvement of reactive oxygen species in the mechanism of action of chloroquine has been supported by the finding that the activity of chloroquine against P. falciparum is enhanced in the presence of H₂O₂.
peroxidative damage to membranes or enzymes is the final target of chloroquine action, this will explain the irreversible nature of chloroquine activity against the parasite, *P. falciparum*.

It has been demonstrated that chloroquine has a significant effect on antioxidant systems in the blood and that oxidative stress/damage is induced in the blood by the drug within 24 h but reaches the peak at about 6 h of oral drug administration. The possible effects of this on the mechanism of action of the drug and its efficacy needs to be further investigated.

REFERENCES