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The influence of chloroquine administration on antioxidant levels, oxidant marker and total cholesterol in Wistar rats

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Abstract
This study was undertaken to determine some biochemical changes of importance associated with chloroquine (CQ) treatment, using rat model of *Plasmodium beighei berghei* parasites. Chloroquine phosphate (5mg/kg body wt, dissolved in distilled water) was administered 3days per week for 8 weeks after infection by *Plasmodium beighei beighei.* The chloroquine did not potentiate any adverse effect on the formation of ascorbic acid-linked lipid peroxidation. The drug increased glutathione contents, but decreased protein and cholesterol levels. Chloroquine alone had no significant effect on malondialdehyde and alanine/aspartate transaminases, but decreased protein synthesis and cholesterol level. The characteristic biologic effects of CQ including the decrease in total cholesterol, protein synthesis, and stabilizer of lipid peroxidation as measured by malondialdehyde and the increased level of glutathione, may enhance the environmental measures of public health in cholesterol compromised individuals.

Keywords: Chloroquine, cholesterol, hypercholesterolemia, roll-back malaria, public health, ascorbic acid-linked lipid peroxidation, hepatic transaminases.

Introduction

Chloroquine (CQ) has become increasingly ineffective largely because of development of resistance to the drug by malaria parasites. Massive use of this drug to help eradicate malaria worldwide backfired when under selective pressure, drug-resistance strain of *Plasmodium falciparum* malaria emerged that gradually flourished (Wellems et, al., 2001, Medilinks 2002, and WHO/UNICEF 2003). The declining therapeutic effectiveness of chloroquine and some other older antimalarial drugs prompted the introduction of more based effective newer drugs such as the artemisinin-combination therapy as well as the concept of roll-back malaria (Li et. al., 2003 and Yamey 2004).

A substantial use of chloroquine is essential to meet the social and economic goals of the endemic community and today's best practice demonstrates that it can be used widely in a cost-effective manner and with a high degree of safety. However, a great deal remains to be done to ensure environmental health and management of chloroquine, within the principles of potential application and sustainable development and improved quality of life for humankind. Two of the major problems, particularly in developing countries, are (a) lack of sufficient scientific information for the assessment of risks entailed by regular use of chloroquine and (b) lack of resources for assessment of chloroquine for which data are at hand.

In hypothetical situation, where CQ resistance to malaria infection has a multigenic mechanism as widely believed (Schwartz 2003), this association may govern other mechanism that has potential benefit in cholesterol reduction (Dushkin et. al., 1986, Beynen 1986 and Achudume and Eno 2000). Bioaccumulation and toxicity of chloroquine in lysosomal cells and liver are well documented (Korolenko et. al., 1990). Our previous investigations showed that chloroquine deaccelerated NADPH-linked lipid peroxidation (Achudume et.al., 1997). Similar reports showed that CQ causes alterations in lipid metabolism (Achudume et. al., 1998) and inhibition of cholesterol synthesis in freshly isolated rat hepatocyte (Sewell et. al., 1983). Taking together, these results provide strong evidence that CQ may still be valid as a drug for other indications.

In the present study, using Ascorbic acid-linked lipid peroxidation, cholesterol, glutathione (GSH) and hepatic enzymes [alanine (ALT) and aspartate (ASP) transaminases] as indices of toxicity, exposure to low-level CQ was investigated as to whether it has potential to ensure environmental health measure for

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cholesterol compromised individuals. Many workers had attempted dietary approaches (DASH) to lower cholesterol level which may reverse hypertension (Obarzanek et. al., 2001, Ignatus et al 2006). Other study used Satin for the treatment of cholesterol (Caspard 2006) and all these treatments have their drawbacks. They either have long-term cardiac risk or elevate liver enzymes and cause myopathy as well as other side effects. None had studied the prophylactic effects of CQ on lipid peroxidation, cholesterol, GSH and liver enzymes to justify calls for public health strategies to maximize the useful life of CQ. In the present study, an assessment of chloroquine as alternative to malaria treatment were determined by measuring the levels of antioxidant (GSH) and oxidant marker (MDA), and total cholesterol levels by analyzing percent parasitemia to the liver enzymes in infected rats.

Materials and Methods

Animals

Adult male albino Wistar rats weighing between 190-200g were obtained from animals holding, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. Animals were housed in ventilated group aluminum boxes (41x28x15 cm) and illumination (12h light cycle starting at 6AM) for at least 7 days before experiments. Animals were maintained on Laboratory chow (Ladokun Feed, Nigeria LTD) and water. Rats were deprived of food for 24h but given free access to water up to the beginning of the study. Protocols describing the use of rats were approved by the Animal Care Committee of OAU, Ile-Ife and in accordance with the American Physiological Society's "Guiding Principles for Research Involving Animals and Human Beings".

Test procedures

All chemicals used were of analytical grade. Chloroquine phosphate (5mg/kg body wt, dissolved in distilled water) was administered orally for 3days before and after infection, i.e. 3 days per week for 8 weeks. The experimental animals received a standard inoculum of $1x10^7$ *Plasmodium beighei berghei* infected erythrocytes intraperitoneally (ip), following the methods of Makinde et. al. (1993). Blood schizontocidal activity (Rane test) was evaluated after 3, 5, and 8 days post infection. This was monitored by collection of drops of blood from the tail vein, followed by fixation, permeability, staining and analyzed with a visible range flow cytometer (Barkan et. al., 2000). The percentage suppression of parasitaemia was calculated for each day by comparing the parasitaemia in infected control with those of treated rats (Ayaiyeola et. al., 2006). The positive control received infected erythrocytes and negative controls were given 0.95 NaCl. At test termination, animals were sacrificed by decapitation. Livers were dissected out and homogenized in TRI-R-STIR-R model K-43, and centrifuged at 6500g for 10 min.

Analytical Procedures

The supernatant was dialyzed against ice cold distilled water and resuspended in 0.15 M KCl to an approximately final concentration of 20 mg of protein per millimeter (approximately 1g of liver per millimeter (Siegler and Kararinoff 1983). The subcellular fraction was used for the measurement of malondialdehyde by thiobarbiturate. The reaction was initiated by addition of ascorbic acid (Shimada et. al., 1979). Reduced glutathione was determined by using aliquots of liver homogenate (20%) diluted with 5% (w/v) TCA containing 3mM EDTA. After centrifugation, DTNB (5, 5-dithiobis-2-nitrobenzoic acid) was added to the supernatant fractions and absorbance was measured at 412nm as described by Reed (1980) and modified by Liu et. al. (2005). Total cholesterol level was determined by using Liebermann Burechard reagent (Abell et. al., 1962). Protein was determined by Lowry et. al. (1951) method.

Statistical Analysis

An ANOVA followed by Dunnett's test was used to compare treated groups to a control group, after verification of homoscedasticity and equal variances between groups.

Results and Discussion

The effects of CQ administration on ascorbic acid-linked lipid peroxidation, protein contents, glutathione, total cholesterol, alanine and aspartate transaminases is summarized in Table 1. Chloroquine treatment for eight weeks did not potentiate any adverse effect on the formation of malondialdehyde in ascorbic acid-linked lipid peroxidation. Chloroquine alone decreased protein synthesis significantly $P<0.05$ and increased glutathione content. It has little or no effect on the hepatic enzymes and significantly ($P<0.05$) decreased cholesterol level. In a similar manner, Chloroquine with infected blood decreased protein content significantly compared to control $P<0.05$. It increased glutathione level and has no effect on

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Table 1: Effect of chloroquine administration on ascorbic acid-linked lipid peroxidation in subcellular fraction of rat liver

Treatment	Malondialdehyde	Protein	Glutathione	Cholesterol	ALT	ASP
8 weeks	nmol/30 min mg protein	mg/ml	mg/ml	mg/100 ml	mg/protein	
Control	0.12±0.1	13.29±0.62	0.300±0.10	12.65±2.49	0.33±0.020	.30±0.01
CQ plus Infected Blood	0.382±0.5	1.522±0.50[x]	0.843±0.21	2.51±8.[1x]	0.29±0.06	0.29±0.09
CQ alone	0.494±0.41	1.783±0.41[x]	0.735±0.26	2.32±1.6[x]	0.36±0.08	0.34±0.06

[x] Significantly different from control P<0.05

Table 2: Schizontocidal activity of Chloroquine on early infection

Treatment	Dose Mg/kg/day	Average % chemosuppression 3 days	5	8
Control	-	0	0	0
CQ	5	25±1.2	58.6±0.6	86.6±1.3

hepatic enzymes and significantly decreased cholesterol level. The schizontocidal action of CQ assessed its chemosuppressive activity is presented in Table 2.

These studies demonstrated that chloroquine effectively inhibited protein synthesis, reduced cholesterol level while increasing reduced glutathione concentrations. It had no significant effect on ascorbic acid-linked lipid peroxidation and ALT/AST transaminases (Tabe1). Under such conditions, the chemosuppressive activity of Chloroquine was greatly diminished (Table2). The percentage chemosuppression was observed to increase by the day and were consistent with those described in literature (Makinde et. al., 1993). Although the results presented did not relate sensitivity of CQ to resistant factors. The study however, showed that CQ treatment did not show any adverse effect on the formation of

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malondialdehyde in ascorbic acid-linked lipid peroxidation. The decrease in cholesterol levels may be the result of CQ- accelerated excretion of cholesterol through feces, after chloroquine interruption of the entero-hepatic recycling of cholesterol (Wellems et. al., 2001).

From the data presented, CQ administration resulted in a modest impairment of cholesterol reabsorption and in a possible alteration in binding and permeability properties of various membrane systems resulting in excretion of proteins. Several studies have shown that CQ binds quantitatively to membrane system and reduces the binding capacity of the plasma membrane through derangement of the recycling of receptors (Wellems et. al., 1991).This result is an extension of the observation of Sewell (1983) and Achudume (1997), that cells exposed to CQ take up and concentrate this drug, resulting in an extensive vacuolation of serum and hepatic transaminases. Furthermore, the effect produced by CQ metabolism on protein synthesis may not entirely due to the products of lipid peroxidation, since, CQ is effectively scavenged by glutathione. GSH deceased the stimulatory action of ascorbic acid-linked lipid peroxidation, in consequence, effectively prevents protein synthesis. Accordingly, the present study having clearly showed the reduction of cholesterol level as a result of CQ ingestion and the high levels of glutathione, CQ may find some uses in individuals with history of excessive accumulations of cholesterol. Though chloroquine may be a resistant drug in malaria infection, it is nevertheless useful in cholesterol intervention.

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