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Effect of Co-administration of Chloroquine Phosphate and Cefuroxime Axetil on Serum Lipids and Lipid Peroxidation in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OEE designed the study and wrote the protocol. Authors MIA and OEE carried out all laboratories work and performed the statistical analysis. Author EJA supervised the work and managed the analyses of the study. Author UAA wrote the first draft of the manuscript. Author UAA managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

There is scanty information on the response of biochemical system to the co-administration of anti-malarial drugs and antibiotics. This was what informed the need to undertake the present study. The effects of co-administration of chloroquine (CQ) and cefuroxime (CE) on serum lipids and lipid peroxidation in albino Wistar rats was investigated. Standard reagent kits were used for the assay. The study included assay for the effect of the co-administration of CQ and CE on high density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and lipid peroxidation. The result revealed that the level of HDL-C was not affected by the co-administration. Those of TC, TG and LDL-C increased significantly ($p<0.05$) above the value obtained for the control group. Malondialdehyde (MDA) level increased significantly ($p<0.05$) in group treated with CQ in combination with CE. The present study has

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revealed that co-administration of chloroquine and cefuroxime tends to raise TC, TG, LDL-C and MDA levels. The result reveals that co-administration of chloroquine and cefuroxime may adversely affect the metabolism of lipids.

Keywords: Chloroquine; cefuroxime; serum lipids; lipid peroxidation.

1. INTRODUCTION

It is well established in the literature that bacterial infections can coexist with severe malaria moreover especially in the tropics [1]. It is advocated that despite appropriate anti-malarial therapy, these patients should receive broad spectrum antibiotic. Moreover in malaria endemic areas, children often carry falciparum malaria asymptomatically, so malaria is over-diagnosed at the expense of other infectious conditions [2]. A study carried out to access the coexistence of bacterial infection with *Plasmodium falciparum* revealed that blood slide positive for *Plasmodium falciparum* had also shown an invasive bacterial disease, and improved diagnosis and treatment of an invasive bacterial disease along with malarial disease are needed to reduce childhood mortality.

Typically, several studies have been carried out to establish the co-infection of *Plasmodium falciparium* and *Salmonella typhi* in different countries [3,4]. Co-infection with malaria and typhoid fever is considered to be common, especially in the tropics where malaria is endemic.

It is also common place in recent times that at the end of malarial therapy therefore symptoms which may or may not be of malarial origin seem to persist. Reasons for these persistent symptoms may not be known until proper medical laboratory tests are conducted. However in an attempt to solve the problem, certain antibiotics are administered alongside anti-malarials. This co-administration ultimately eradicates the infections (bacterial or parasitic). It is not well established or known whether the drug combination is safe to the biochemical system or not.

Independently, chloroquine (an anti-malarial therapy) has been reported to increase low density lipoprotein removal from plasma in systemic lupus patients and cefuroxime (an antibiotic) has also been reported to induce coronary artery spasm which manifest as kounis syndrome [5,6]. However, there is scanty information on the response of the biochemical system to the co-administration of anti-malarial and antibiotic agents. Therefore this study was imperative as the effects of the co-administration of anti-malarial and antibiotic agents on the albino Wistar rats will be investigated. Specifically, the effect of chloroquine and cefuroxime co-administration on lipid profile and lipid peroxidation in albino Wistar rats is investigated.

Serum lipid profile is implicated in cardiovascular disorders such as atherosclerosis, coronary heart disease, etc. High cholesterol is one of the risk factors involving many cardiovascular diseases. Blood LDL cholesterol, bad cholesterol, carries cholesterol to tissues, including coronary arteries and develops thickening of artery walls. The higher cholesterol level, the greater the risk of atherosclerosis, hypertension, coronary heart disease and heart attack. This study will give an insight into modulation of lipids during administration of these drugs.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Antimalarial: Chloroquine phosphate (250 mg)

Chloroquine phosphate (250 mg) is manufactured by Evans Medical PLC (RC 1161), Km 32, Lagos – Badagry Expressway, Agbara Industrial Estate, Agbara, Ogun State, Nigeria.

2.1.2 Antibiotic: Cefuroxime axetil tablets (500 mg)

Cefuroxime axetil tablet (500 mg) is manufactured by Okasa Pharma PVT Ltd, L-2 Additional MIDC Area, Satara 415004- India. It is manufactured for CIPLA Ltd India under loan licence. It marketed by Evans Medical Plc, Agbara Industrial Estate, Agbara, Ogun State, Nigeria.

2.2 Experimental Design and Treatment of Animals

A total of twenty four (24) albino Wistar rats (male and female) were weighed with average

weight of 151 g and divided into four (4) different groups of six (6) per group and put into rat cages. The rats were maintained under hygienic and favourable conditions under a 12 hour light / 12 hour dark cycle with feeds and water provided *ad libitum*. Group 1 rats were given normal rat pellets and water. Group 2 rats were treated with 4.17 mg/kg body weight (bw) of chloroquine (CQ). Group 3 rats were administered concomitantly with 4.17 mg/kg body weight (bw) of chloroquine and 8.33 mg/kg bw of cefuroxime (CE). Group 4 rats were treated with 8.33 mg/kg bw of cefuroxime (CE) only. The drugs were dissolved in distilled water and administered orally with the aid of an oral cannular to the experimental rats. Chloroquine was administered for three days while cefuroxime was administered for seven days. 24 hours after the last administration, the animals were sacrificed under chloroform anaesthesia, and blood samples were collected through cardiac puncture into labeled sample bottles. Serum was obtained from the whole blood by centrifugation (Table centrifuge by B. Bran Scientific and Instrument Company, England) at 3000 rpm for 10 minutes and used for biochemical assay.

2.3 Estimation of Biochemical Parameters

Estimation of high density lipoprotein-Cholesterol (HDL-C) Level was carried out according to the method of Lopes Virella et al. [7]. Low density lipoproteins (LDL and VLDL) and chylomicrons fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined. Estimation of Serum Cholesterol (TC) level was carried out according to the method of Allain et al. [8]; Roeschlaw et al. [9]. The concentration of cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator, quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase [10]. Estimation of Serum Triglycerides (TG) level was carried out according to the method of Tietz [11]. The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4 – aminophenazone and 4 – chlorophenol under the catalytic influence of peroxidase. Low density lipoprotein-Cholesterol (LDL-C) was carried out according to the method described in Teco Diagnostic direct LDL-cholesterol Reagent product leaflet although the Friedwald formula can also be used [12].

Assessment of Lipid Peroxidation (LPO) was carried out according to the method of Esterbauer et al. [13]. Assessment of lipid peroxidation is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole (RI) with malondialdehyde (MDA) and 4-hydroxyalkenals at 45℃. One molecule of either MDA or 4-hydroxyalkenals reacts with 2 molecules of R1 to yield a stable chromophore with maximal absorbance at 586 nm. For simultaneous estimation of MDA concentration and 4-hydroxyalkenals, one must use the procedure utilizing methanesulfonic acid (MSA) as the acid solvent. The procedure in which HCl is used will only detect MDA, since the 4-hydroxyalkenals does not form a chromophore with reagent R1 under those conditions.

2.4 Statistical Analysis

Data obtained were analysed using appropriate statistical tool such SPSS statistics, analysis of variances (ANOVA), when significant, Kruskal-Wallis test was used for non parametric analysis, student and Mann-Whitney test were carried out on the data when only two groups are compared. Results were considered significant at ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Results

The result of this study is presented in Tables 1 and 2. The LDL concentration increased significantly ($p < 0.05$) when compared to the control while HDL cholesterol showed no significant modulation as a result of the drug treatment. TG was ignificantly increased across treatment groups while total cholesterol was increased significantly only in group treated with chloroquine. Serum MDA was significantly increased in group treated with both drugs simultaneously.

3.2 Discussion

High prevalence of malaria and typhoid fever in the tropics has made co-infections common. However, the actual and precise underlying mechanism to explain the association between malaria and salmonellosis is still uncertain [14].

The treatment of malaria and typhoid co-infection is a common phenomenon in many parts of Africa [15]. This is because malaria and typhoid fever have been associated with poverty and

underdevelopment with significant morbidity and mortality. An association between malaria and typhoid fever was first described in the medical literature in the middle of the 19th century, and was named typhomalarial fever by the United States Army [16].

It has been advocated that despite appropriate anti-malarial therapy, these patients should receive broad spectrum antibiotics. Moreover, in malaria endemic areas, children often carry falciparum malaria asymptomatically, so malaria is over diagnosed at the expense of other infectious conditions.

Serum lipid profile in the treated Wistar rats is presented on Table 1. Cholesterol is a fatty substance found in blood, bile and brain tissue. It serves as a precursor for the synthesis of bile acids, steroids, and vitamin D. The determination of serum cholesterol is a major aid in the diagnosis and classification of lipidemia [11]. Other conditions such as hepatic thyroid diseases influence cholesterol level [17]. They act as functional and structural components of bio-membranes and form insulation to allow nerve conduction and prevent heat loss.

High density lipoproteins (HDL) are one of the major classes of plasma lipoproteins. They are composed of a number of heterogenous particles, including cholesterol and vary with respect to size and content of lipid and apolipoprotein. HDL serves to remove cholesterol from the peripheral cells to the liver, where the cholesterol is converted to bile acids and excreted into the intestine. The apparent fixed level of HDL in the present study literally implies that HDL level was not affected by the drugs treatment. Rahilly [18] advocated that for a fixed level of HDL, the risk of heart disease increases three-folds as LDL varies from low to high.

Triglyceride measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders e.g. diabetes mellitus, nephrosis and liver obstruction. In the human body, high levels of triglycerides in the blood stream have been linked to atherosclerosis (hardening of the arteries), and by extension the risk of the heart disease and stroke [19].

Total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-C) increase significantly ($P<0.05$) as shown in Table 1 when the group treated with CQ was compared to control contrary to a study by Sachet et al. (2007) where chloroquine was reported to increase removal of LDL-cholesterol from plasma in lupus patients. Triglycerides (TG) and LDL-C also increased significantly ($p<0.05$) when the group treated with CQ+CE and the group treated with CE alone were compared to the control, an indication of increased risk of heart disease.

An inverse relationship between HDL-cholesterol (HDL-C) levels in serum and the incidence / prevalence of coronary heart disease (CHD) has been demonstrated in a number of epidemiological studies. The importance of HDL-C as a risk factor for CHD is now recognized [20].

Data from the Landmark Framngham Heart Study revealed that, for a given level of LDL, the risk of heart disease increases 10-folds as the HDL varies from the high to low. On the converse, however, for a fixed level of HDL, the risk increases 3-folds as LDL varies from low to high [18].

Malondialdehyde reacting substances and serum in treated Wistar rats were shown on Table 2. Lipid peroxidation, a known mechanism of cellular injury is a chain reaction initiated by free radicals. It is an index of oxidative stress in cells and tissues. The free radical oxidation of lipid molecules proceeds from the fatty acylmethylene group adjacent to a double bond [21] to form lipid hydroperoxides which are capable of further reactions to yield various classes of peroxides. Lipid peroxides are unstable and decompose to form series of complex products including reactive carbonyl compounds. Polyunsaturated fatty acids on oxidation decompose to malondialdehyde (MDA) and 4 – hydroxyalkenals (HAE). The levels of these products in serum or tissues have been widely accepted as a measure of lipid peroxidation [13]. Several studies in literature have suggested that drugs used to treat malaria, such as chloroquine lead to oxidative stress, particularly in the erythrocytes through generation of intracellular reactive oxygen species (ROS) causing lipid peroxidation [22].

Biological membranes which contain phosphor-lipids rich in polyunsaturated fatty acids are continuously being oxidised by superoxides, hydroperoxyl and peroxyl radicals emanating from exposures to radiations and oxidative reactions involving endogenous and exogenous compounds in the body [23].

Table 1. Serum lipid profile of albino Wistar rats co-administered with 4.17 mg/kg bw of CQ and 8.33 mg/kg bw of CE

Treatment	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
CTR	1.84±0.05	5.90±0.16	0.58±0.01	0.30±0.09
CQ	2.28±0.16*	6.91±0.24*	0.54±0.01	0.39±0.12*
CQ+CE	1.79±0.08**	6.45±0.09*	0.57±0.04	0.57±0.09*
CE	1.66±0.072**	6.49±0.06*	0.54±0.01	0.75±0.09*

CQ = Chloroquine, CE = Cefuroxime

** Significantly different from control (CTR) (P <0.05), ** Significantly different within group, number of rats (n) = 6*

The toxicity of lipid hydroperoxides to animals is best illustrated by the lethal phenotype glutathione peroxidase 4 (GPx4) knockout mice. These animals do not survive past embryonic day 8, indicating that removal of lipid hydroperoxides is essential for mammalian life [24].

Table 2. Effects of the co-administration of 4.17 mg/kg bw of CQ and 8.33 mg/kg bw of CE on lipid peroxidation in albino Wistar rats (assessed in terms of MDA level)

Treatment	MDA (µmol)
CTR	4.76±0.36
CQ	3.93±0.41**
CQ+CE	5.97±0.30*
CE	4.28±0.22**

`CQ = Chloroquine, CE = Cefuroxime

** Significantly different from control (CTR) (P <0.05),*

*** Significantly different within group, number of rats (n) = 6*

Data from the present study, revealed a significant increase ($p<0.05$) in MDA concentration in the group treated with CQ+CE compared to the control. The result is an indication of potential toxicity to membrane by the drug combination though the level of toxicity noticed does not seem to trigger the antioxidant defense system observed in this study. These systems include antioxidant enzymes, transport and storage proteins, etc. Oxidative stress which is associated with cellular injury in many pathological conditions occurs when there is an imbalance between free radical generation and radical scavenging systems [25].

4. CONCLUSION

From the results of this study, it can therefore be concluded that the co-administration of chloroquine and cefuroxime may adversely affect the metabolism of lipids.

ETHICAL APPROVAL

Ethical approval for the study was obtained from the Ethical Committee of the College of Health Sciences, University of Uyo, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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