

## Effects of Incremental Doses of Chloroquine Phosphate on the Formed Elements of Blood.

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**Abstract:** Easy access to chloroquine (CQ) in many developing countries can result to intake of inappropriate doses. This study was therefore conducted to evaluate the acute effects of high doses of CQ on hematological parameters and plasma Na<sup>+</sup> and K<sup>+</sup> concentrations. To do this, Swiss albino rats were administered incremental doses of CQ intraperitoneally at dose rates ranging between 0.5 to 8mg/kg b.wt. Two hours later, blood samples were taken and analysed. 8mg/kg b.wt of CQ induced significant decrease in red blood cells (RBC), hemoglobin (Hb) and significant increase in mean corpuscular volume (MCV). There was a significant increase in white blood cell count at all dose levels. Packed cell volume (PCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were not affected by all dose ranges. There was significant increase in plasma Na<sup>+</sup> and K<sup>+</sup> by dose rates of 4 and 8 mg/kg but not by lower doses. These data indicate that most CQ induced toxicities on the formed elements of blood occurs at doses greater than 4 times the standard therapeutic dose in rats.

*key words:* chloroquine, toxicity, blood, rats

### INTRODUCTION

Malaria infection can be characterised by leucopenia, lymphopaenia and thrombopaenia (Nicolas et al, 1997) as well as the digestion of host erythrocytes' hemoglobin by parasites (Sherman, 1979, Slater, 1992, and Roth, 1989). Chloroquine is one of the most widely used drugs for the treatment of malaria (Yayon et al, 1984, Ndyomugenyi and Magnussen 1997). However, several toxic effects of CQ have been reported including neutrophilic toxicity (Naisbitt, et al, 1997), myo and neurotoxicities (Estes, et al, 1987, Ochsendorf & Runne 1991) and retinopathy (Ochsendorf & Runne 1991). Data regarding CQ toxicity on blood cells which

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habours the plasmodium parasites are scanty. The need to evaluate the possibility of toxicity of CQ on parasite-free cells is therefore vital. This will establish whether in addition to parasitic effects, CQ induces deleterious effects of its own. Pharmacokinetically, CQ is rapidly absorbed following parenteral injections, and widely distributed in body tissues (Aderomu et al, 1986, Krishna and White 1996) and so toxicity can be rapid. High concentrations are obtained in red blood cells (Salako & Adeluis 1983). Uncontrolled use of CQ which occurs in developing countries (Odoi et al, 1995, Ndyomugenyi & Magnussen, 1997) can be a source of great danger (Ochsendorf & Runne 1991). This study was therefore conducted to examine the hematological and plasma Na<sup>+</sup> and K<sup>+</sup> alterations following acute in vivo exposure of adult rats to incremental doses of CQ. This is in regard to the fact that the study of drug effects on cellular constituents of blood has not been a standard part of routine investigations performed in screening of drugs for potential toxic effects (Hinderling, 1997).

## MATERIALS AND METHODS

### **Animals**

Thirty five Swiss Albino rats of mixed sex, mean body weight  $170 \pm 12$ g and aged 6 months were used in this study. They were purchased from the Department of Veterinary Physiology and Pharmacology, University of Ibadan. They were housed in cages in a insect-proof house and provided with commercially prepared rat diet (21% protein, 3.5% fat, 6% fibre, 0.8% calcium, and 0.8% phosphorus, Ladokun Feeds Nig. Ltd, Ibadan). Animals were randomly allocated to five experimental groups (I, II, III, IV and V) of 5 rats each while group VI (n=10 served as control). Care was taken to ensure that all animals were treated in conformity with the standard animal ethics and experimentation guidelines.

### **Drug preparation and administration**

Chloroquine phosphate was obtained in 5ml ampoules from KRKA, Novo Mesto Yugoslavia. Each ml of injectable solution contained 64.5mg chloroquine phosphate (equivalent to 40mg chloroquine base). The required dose was further diluted with physiological saline and volume made up to 1ml followed by intraperitoneal injection. Rats in groups I, II, III, IV and V received an incremental dose of 0.5, 1, 2, 4 and 8mg/kg of the CQ base by intraperitoneal injection. The dose administered to group II was calculated from the dose given to group I (0.5mg/kg) by arithmetically multiplying it by 2. Successive doses were calculated in similar manner by multiplying the dose administered to the preceding group by 2. Group VI received equal volume of physiological saline. Rats were returned to their cages.

### **Blood collection and analyses**

Two hours post injection, each rat received an intraperitoneal injection of pentobarbital (Nembutal, 50mg/kg, Abbot laboratories, USA) to induce anaesthesia in order to minimise pain during the experimental procedure. After rats have been fully anaesthetised, blood sample was collected by cardiac puncture. The collected blood was immediately transferred into

a sample bottle prepared with ethylene diamine tetracetic acid (EDTA). Determination of haematological parameters was carried out within 1 hour of blood collection. For electrolyte analysis, plasma was separated by centrifugation. Fresh plasma was then transferred into polypropylene tubes and frozen at -70°C until analysed. Packed cell volume (PCV) haemoglobin (Hb), white blood cells (WBC) and red blood cells (RBC) were determined using standard methods. Erythrocyte indices were estimated as proposed by Ganong (1995). Serum Na<sup>+</sup> and K<sup>+</sup> were determined by flame photometry (Gallenkamp, London).

### Statistical analysis

Data are expressed as mean values  $\pm$  SEM. Statistical inhomogeneity between experimental and controlled measurements were analysed by ANOVA using the statistical package StatView 4.5 (Stat View, Abacus Corp, USA 1992–1994).

## RESULTS

The data indicate that 8mg/kg CQ produced significant decrease in RBC, Hb, and increase in mean corpuscular volume (MCV) over the control values. All doses ranging between 0.5 and 8mg/kg produced significant increase in WBC counts over the control values. Packed cell volume (PCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were not affected by all dose ranges (Table 1). There was significant increase in plasma Na<sup>+</sup> with drug doses at 4mg/kg and 1.28mg/kg. Doses between 0.5 to 2mg/kg did not induce any significant change in plasma Na<sup>+</sup> concentration (Fig. 1). Plasma K<sup>+</sup> was significantly increased by drug doses of ranging between 2 to 8mg/kg but not by doses ranging between 0.5 to 1mg/kg (Fig. 2). Death was recorded in one rat receiving 8mg/kg.

Hematological Parameters	Experimental group/drug dosage (mg/kg b.wt)					
	I (0.5)	II (1)	III (2)	IV (4)	V (8)	VI(Control)
PCV (%)	45.2 $\pm$ 0.4	44.8 $\pm$ 0.5	44.8 $\pm$ 0.4	45 $\pm$ 0.4	45.4 $\pm$ 0.2	44.8 $\pm$ 0.2
RBC ( $\times 10^6/\text{mm}^3$ )	6.7 $\pm$ 0.04	6.4 $\pm$ 0.06	6.4 $\pm$ 0.06	6.4 $\pm$ 0.04	5.7 $\pm$ 0.05**	6.4 $\pm$ 0.04
WBC ( $\times 10^3/\text{mm}^3$ )	5.7 $\pm$ 0.05***	5.2 $\pm$ 0.06**	5.4 $\pm$ 0.08***	5.3 $\pm$ 0.09**	5.3 $\pm$ 0.07**	4.8 $\pm$ 0.07
Hb (g/dl)	12.6 $\pm$ 0.05*	12.8 $\pm$ 0.11**	12.8 $\pm$ 0.14	12.1 $\pm$ 0.05***	12.9 $\pm$ 0.07	13.2 $\pm$ 0.1
MCV (fl)	66.6 $\pm$ 0.5	66.0 $\pm$ 0.7	66.4 $\pm$ 0.5	68.8 $\pm$ 1.1	78.8 $\pm$ 0.6***	68.4 $\pm$ 1.3
MCH (pg)	18.8 $\pm$ 0.4	20.0 $\pm$ 0.7	19.4 $\pm$ 0.5	19.6 $\pm$ 0.5	20.2 $\pm$ 0.4	18.8 $\pm$ 0.4
MCHC (%)	27.8 $\pm$ 0.4	28.4 $\pm$ 0.5	27.8 $\pm$ 0.4	26.4 $\pm$ 0.4	25.8 $\pm$ 0.4	26.6 $\pm$ 0.4

**Table 1.** Hematological changes following the administration of increasing doses of chloroquine.

Test group statistically significant at \*P<0.01, \*\*P<0.001 and \*\*\*P<0.0001 compared to control.

PCV=packed cell volume, RBC=red blood cells, WBC=white blood cells, Hb=hemoglobin, MCV=mean corpuscular volume, MCH=mean hemoglobin concentration, MCHC=mean corpuscular haemoglobin concentration

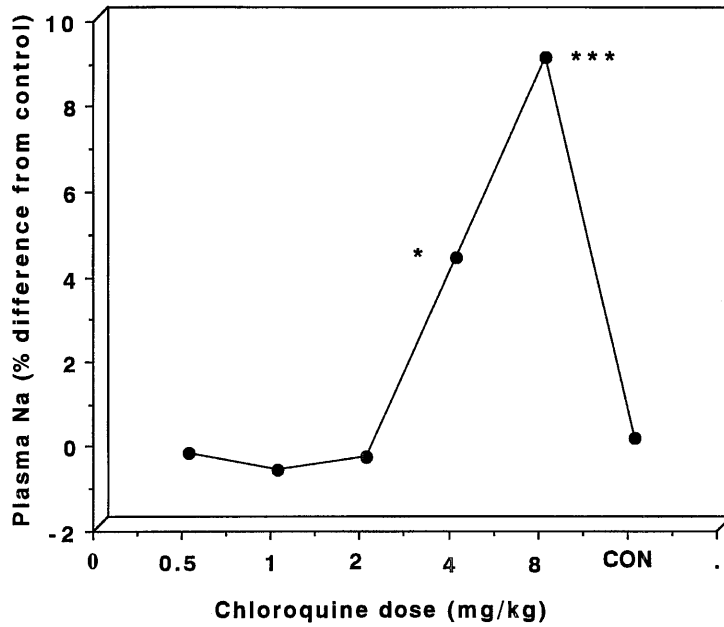


Fig. 1. Plasma sodium changes following the administration of increasing doses of chloroquine. Test group statistically significant at  $^*(P<0.01)$ , and  $^{***}P<0.0001$  compared to control.

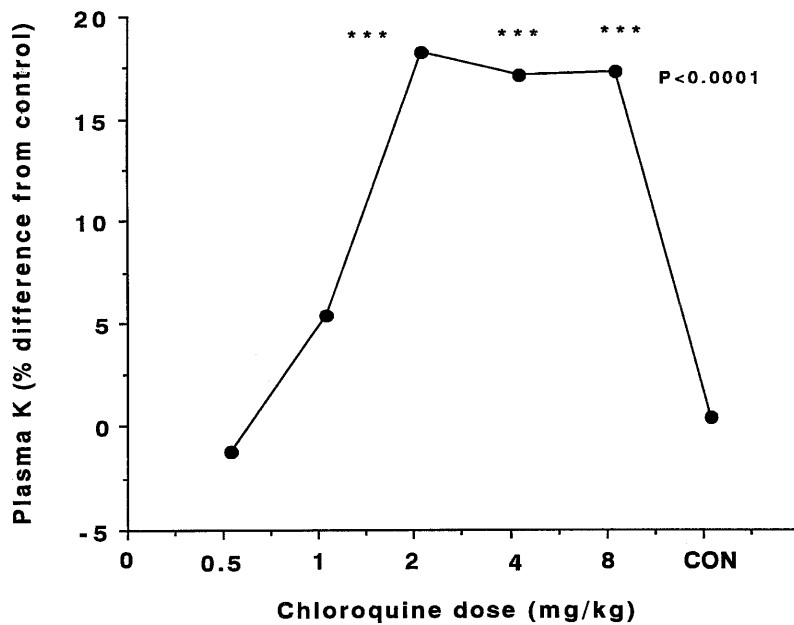


Fig. 2. Plasma potassium changes following the administration of increasing doses of chloroquine. Test group statistically significant at  $^{***}P<0.0001$  compared to control.

## DISCUSSION

One of the prominent changes noticed following the administration of CQ on hematology is the increase in WBC count. Administration of the therapeutic as well as higher doses resulted in an increase in WBC, even though the increase was not in a strictly dose dependent manner. Because no differential leucocyte count was performed, it was not possible to determine whether the increase was due to lymphocytes or neutrophils. However, previous study had shown that polymorphonuclear (PMN) leucocytes accumulates CQ more than mononuclear leucocytes and red blood cells. This was attributed to the acidic cellular organelles such as lysosomes contained in the PMN Leucocytes which trap the weak base CQ (Raghoobar et al, 1986), and the accumulation of CQ in leucocytes is both dose and time dependent (French et al, 1987). The explanation for the increase in WBC count is that since the increase in WBC was detectable as early as 2 hours after CQ injection, the drug might have acted on the bone marrow reserve pool to release mature and immature neutrophils. Although some other reports have shown an increase in leucocyte count following drug administration, these chemicals are not structurally related to CQ. The reports attributed such increase to direct stimulation of the immunological defence system due to the presence of the drug as a foreign compound in the body.

The result also showed that CQ induced no changes in RBC, Hb and MCV at doses below 8mg/kg. This mode of response indicate that induction of toxicity occurred in a dose-dependent manner. A previous study (Agomo et al 1992) found that CQ decreased the numbers of reticulocytes in normal mice. However, they also observed a decrease in the numbers of nucleated cells. Although the mechanisms responsible for the decrease in the RBC counts are not clear, compartmental shifts, hypotension, intravascular haemolysis or splenic sequestration of cellular blood elements are possible causes. Previous studies has shown that ferriprotoporphyrin (FP) IX induced RBC hemolysis is potentiated by CQ. The change in RBC numbers did not affect the PCV because probably this effect was counter-balanced by the increase in MCV.

Even though the cause of death in one rat receiving 8mg/kg was not investigated, it was attributed to CQ toxicity. Previous study have shown that CQ can induced potentially lethal hypotension, especially when given by intravenous injection (Krishna and White 1996).

High doses of CQ can also have some clinical implications since the result of a study in rats showed evidence that febrile illness can contribute to the potentiation of CQ toxicity (Osifo, 1980). There was evidence equally that digestion of hemoglobin by malaria parasites produces FP IX which induces RBC hemolysis and its effect is potentiated by CQ. The possibility exists therefore for an interactive effect with high CQ doses.

The absence of marked changes in PCV with increase in the concentration of plasma electrolyte in response to the high CQ doses may suggest that there was possibly no changes in fluid balance. Several drugs have been reported to induce imbalance in serum sodium and potassium (Brunner & Frick 1968, Feig & McCurdy 1977, Lawson, 1981). Some of the mechanisms suggested for such changes in the absence of fluid shifts is alteration of plasma membrane integrity which can result to changes in permeability. The significant increase in plasma Na<sup>+</sup> and K<sup>+</sup> observed may suggest impaired ability of erythrocytes to maintain cation gradients through membrane perturbing at these high doses resulting to increased permeability of cell membrane.

The overall result indicate that high doses of the CQ can affect cellular composition of the blood through alteration of blood cell numbers.

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