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# Effects of Chloroquine on the Morphology and Stereology of Some Tissues in Sprague-Dawley Rats

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ABSTRACT: Significant binding of chloroquine occurs in the liver, kidney and spleen hence this study was designed to determine the effects of administration of chloroquine on the morphology and stereology of the liver, kidney and spleen. Ten rats were exposed to chloroquine once a day for three days. The treated rats received the 0.125ml/100g body weight of chloroquine phosphate injection intraperitoneally. Control rats received the same amount of normal saline intraperitoneally. The histology of the chloroquine-treated kidney, liver and spleen was also compared with controls. It was observed that chloroquine caused malformation in these tissues. Histologically, the micrographs of control and treated rats, liver, kidney and spleen were compared. Investigations confirmed defects in microscopic structures, e.g. for the kidney there were few renal corpuscles in the treated rats compared with controls. For the liver there were few blood vessels in the treated rats compared with controls. For the spleen there were few white pulps in the treated rats compared with controls with controls. Stereologically, the parameters measured for kidney, liver and spleen was also compared with controls. The estimated absolute volume V =Vv (structure) x v (ref) of the blood vessels, renal corpuscles and white pulps of the fractions were determined and compared. For the liver chloroquine caused a reduction in the absolute volume of the blood vessels when compared with controls. For the kidney, it also caused a reduction in the absolute volume of the white pulps when compared with control rats.

Keywords: Malaria; **C**hloroquine; Liver; Kidney; Spleen; Sprague-Dawley rats; Stereology; Morphology.

## Introduction

Approximately 50-70% of chloroquine in plasma is bound to plasma proteins. The tissues exhibit particularly high binding to chloroquine especially those containing melanin, for example the retina. Significant binding also occurs in the liver, kidney and spleen. Chloroquine (Resochin,Avloclor, Nivaqiune, Arelen) $C_{18}H_{26}CIN_3$ 7- Chloro - 4- (4'- diethlyamino-1'-methylamino0 quinoline. Chloroquine is a white powder with a bitter taste, prepared by chemical synthesis. It is available as sulphate and phosphate salts. The sulphate (1 in 3) and the phosphate (1 in 4) are soluble in water. Chloroquine is best known as an antimalarial agent but it is also used in the treatment of rheumatoid arthritis. Chloroquine is effective against the erythrocytic stages of all four plasmodium species which cause human malaria with the exception of matured *Plasmodium falciparum* gametocytes.

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The exact mechanisms of action of chloroquine against malaria parasites are not fully understood. Parasitized red cells accumulate approximately 100-600 times as much chloroquine. The concentration of chloroquine in malaria parasite requires energy and is thought to require a membrane. There are three theories on the way state as that chloroquine, being a basic compound, is protonated in the lysosomes thus raising lysosomal pH. This effect may raise the intralysosomal pH above a critical level all bring about loss lysosomal function. This would reduce the parasite's digestion of heamoglobin, and thus prevent its growth.

Chloroquine intercalates into double stranded DNA and inhibits both DNA and RNA synthesis. The intercalation theory suggests that chloroquine may be bound with increased affinity by certain parts of the genome and be toxic to the malaria parasite by selective accumulation in specific genes, inhibiting their expression. The ferriprotorphyrin IX (FP) which inhibits sequestration of FP into malaria pigment. This could impair heamoglobin degradation and permits damage to the food vacuole sufficient to discharge its pH gradient, .antimalaria activity is possessed equally by the enantiomers of chloroquine and the main metabolite desethlychloroquine is also active against chloroquine-sensitive Plasmodia.

Chloroquine also has anti- inflammatory activity. The concentrations of chloroquine or hydrochloroquine found in serum in the treatment of rheumatoid disease raise the pH of acid vesicles in mammalian cell within 3-5 min in vitro. This and the observation that the view that chloroquine and hydoxychloroquine act in the rheumatic disease by raising the pH of acid vesicles. Effects of raised vesicle pH include inhibition lysosomal proteolysis, interference with the targeting of acid proteases and inhibition of cellular maturation .raise pH in the macrophage vesicle can interfere with antigen processing. This is thought to be the explanation for the impaired antibody response to pre-exposure to human diploid cell rabies vaccine found in individual receiving concurrent chemoprophyaxis with chloroquine. In addition, chloroquine inhibits the chemotactic response of mononuclear cells and suppresses lymphocytes transformation. It is therefore very important to study the effects of chloroquine on the liver, kidney and spleen.

## Materials and Methods

Twenty female Sprague-Dawley rats were collected from the animal house of the College of Medicine University of Lagos Akoka, Lagos State. They weighed between 100-150g and were fed with normal rat feeds from Pfizer PLC Ikeja Lagos. The weights of the animals were taken twice daily throughout the duration of the experiment. Ten female rats were be used as controls. The remaining ten female rats were labelled by ear puncture as treated rats and kept in cages. Administration of drug was 0.125ml of chloroquine/100g body weight for 3 days intraperitoneally. Chloroquine phosphate injection was obtained from the Community Pharmacy of the Lagos University Teaching Hospital (40mg/ml chloroquine phosphate injection). The control received the same quantity of normal saline.

### Animal Sacrifice

At the expiration of the treatment period, the animals were sacrificed by diethyl ether decapitation and the liver, kidney, and spleen were removed for morphological and histological assessment.

### Histological Analysis

The twenty male rats were sacrificed after treatment with the chloroquine phosphate injection .The liver, kidney and spleen were removed and fixed in Bouin's fluid. Each specimen of equal length was cut transversely and longitudinally into serial cross sections of 3µm normal thickness with Reichert Jung Supercut Mictrotome for control and treated rats. The tissues were sectioned using tissues preparation method with heamatoxylin and eosin stains and examined the light binocular microscope at a magnification of 100 and 400 respectively.

**Stereological Analysis**

The vertical sections of the histochemical preparation of stratum length of 0.5cm from 10 control and 10 treated rats liver, kidney, and spleen were made at a final print magnification of 100 and 400 respectively. Five slides were obtained from the control and 5 slides from the treated rats.

For each of the fractions, the N/A ≡ number of blood vessels, renal corpuscles, per unit area of the fractions were estimated by point counting method using the forbidden rule (Hans Gundersen, 1977) which states that any structure that touches the forbidden line must not be counted. The reference volume V(ref) of blood vessels, renal corpuscles and white pulps were estimated by point counting (Wiebel, 1979, Gundersen *et al*, 1988).

At Magnification (M) = 100 final magnification using a *Square Grid* of test point diameter (d) =1.2cm apart. The test system used in the light microscopic analysis within a square frame measuring 20cm x 20cm onto which microscopic image was projected using a wild leitz microscope equipped with a mirror at a magnification of 25 on a white screen.

Estimated V(ref) = (stratum length) x $\frac{d^2}{M^2}$ x mean N/A (structure).

d= diameter of test grid
M=magnification of projection

The relevant volume density of blood vessels, renal corpuscles, and white pulps of the fractions Vv (structure) were estimated on the same section at a final magnification of 100. Each field was projected onto a test system consisting of three sets of points with numerical densities in the ratio 1:4:16. The corresponding distance between the test points of each set were 4.8, 2.4 and 1.2cm respectively.

The criteria for test point design and allocation were based on efficiency considerations; thus approximately the same number of test points (which does not need to exceed 200) should be in each structure within each organ (Gundersen and Jensen, 1987; Gundersen *et al* ., 1988; Cruz Orive and Wiebel; 1990). The required volume density of the fractions were estimated as follows:

**Estimated Vv(structure) = $Nv_R$ x N/A( structure)**
**Vv = volume density $Nv_R$ = numerical density ratio**

Finally, the absolute volume of blood vessels, renal corpuscles and white pulps within each organ was estimated using the following equation:

**V(structure) =Vv (structure) x V (ref)**
V(structure) = absolute volumes of structure
Vv(ref) = Reference volume of structure

**Statistical Analysis**

Statistical analysis was carried out using Student t- test.

## Results and Discussion

**Histomorphometric Effects**

Chloroquine caused defects in the microscopic structure of the liver kidney and spleen of the Sprague-Dawley rats. Renal corpuscles were few and deformed with noticeable patches in the kidney when compared with controls. Blood vessels in the treated rats compared with controls were few in the liver when compared with controls (Table 1). They were few white pulps in the treated rats spleen compared with controls. Stereologically, the individual estimated absolute volume of fractions were determined and compared. For the liver there was a reduction in the absolute volume of the blood vessels when compared with controls. For the kidney, Chloroquine caused a reduction

in the absolute volume of the renal corpuscles when compared with controls. Lastly for the spleen chloroquine caused a reduction in the absolute volume of the white pulps when compared with controls.

**Table 1: Estimated absolute volumes ($cm^3$) of blood vessels renal corpuscle and white pulps of chloroquine treated and control rats**

| TISSUE | Control Rats (n=10) | Chloroquine Treated Rats (n=10) |
|---|---|---|
| Liver (blood vessels) | $4.76 \times 10^{-3} \pm 0.19^a$ | $1.93 \times 10\text{-}3 \pm 0.95^b$ |
| Kidney (renal corpuscles) | $4.20 \times 10^{-3} \pm 0.02^a$ | $4.15 \times 10\text{-}3 \pm 0.18^b$ |
| Spleen (white pulps) | $5.65 \times 10^{-3} \pm 0.48^a$ | $4.0 \times 10\text{-}3 \pm 0.10^b$ |

Figures represent the mean±S.E.M
Values that have difference superscripts in the same row are significantly different ($p<0.05$).

**Table 2: Mean number of blood vessels per unit area (N/A)**

| GROUP (n=20) | MEAN (N/A) |
|---|---|
| CONTROL (CO) | 3.0 |
| TREATED(CQ) | 1.0 |

CO =CONTROL
CQ =CHLOROQUINE TREATED RATS

**Histology**

This study focused on the microscopic structures of the liver, kidney and spleen of animals treated with chloroquine once a day for three days. The investigation confirmed defects in microscopic structures. For the kidney, there were few renal corpuscles with noticeable patches in the treated rats compared with controls .for the liver there were few blood vessels in the treated rats compared with controls. For the spleen there were few white pulps in the treated rats compared with controls (Patricia *et al*., 1981).

**Table 3: Mean number of renal corpuscles per unit area (N/A)**

| GROUP (n=20) | MEAN (N/A) |
|---|---|
| CONTROL(CO) | 2.0 |
| TREATED(CQ) | 1.8 |

CO =CONTROL
CQ =CHLOROQUINE TREATED RATS

**Table 4: Mean number of white pulps per unit area (N/A)**

| GROUP (n=20) | MEAN N/A |
|---|---|
| CONTROL (CO) | 1.5 |
| TREATED (CQ) | 2.0 |

CO =CONTROL
CQ =CHLOROQUINE TREATED RATS

**Stereology**

This study focused on the morphometric investigations. Absolute volumes of the liver, kidney and spleen. Special components were stereologically estimated after treatment with chloroquine. The investigation confirmed that chloroquine has deleterious effects on the quantitative analysis of these important tissues of the body (Ausburger & Arnold 1991). There was a reduction in the absolute volume of the blood vessels present in the liver after treatment with chloroquine compared with controls. There was also a reduction in the absolute volume of the renal corpuscles after chloroquine treatment compared with controls. There was a reduction in the absolute volume of white pulps after chloroquine treatment compared with controls, thereby confirming the report of Cruz-Orive *et al*. (1993).

**Conclusion**

In summary the present study has demonstrated that chloroquine, though an antimalaria drug when taken in the right dosage, has deleterious effects on some vital organs in the body. It also has deleterious effects on the microscopic structures of the liver, kidney and spleen and on the morphometric /quantitative analysis of the liver, kidney and spleen vital components.

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## References

Adelusi S.A *et al* (1982) Protein binding of chloroquine in the presence of aspirin. J. Clin. Pharmacol. 13 (3):451-452.
Asaoka K., Ito H. & Tisell (1980): Morphology of rats prostate lobes and seminal vesicles after long–term estrogen treatment. Acta Pathol Microbial Immunol. Scand. (A) 90:441-448.
Augsburger, H, U. Farber (1991): Morphological basis of urinary continence Anat Hist Embryol 20:265
Author *et al* (1976) Childhood chloroquine poisoning–Wisconsin and Washington. Journal of the American Medical Association 260:1361.
Bunnag, D., Harinasuta, T . (1987): Quinine and quinidine in malaria in Thailand Acta Leidansia 55:163-166.
Charles M; Wool .F (1968) Principles of Biometry 1:1-350.
Charoasia B.D (1980) Human anatomy 3: 200-250.
Cope, G.H (1979) stereological analysis of the duct system of the rabbit parotid gland J.Anat 126:591-604.
Cruz-Orive, L.M. E.R Weibel (1990): Recent stereological methods for cell biology : A brief survey Am J Physio 258: L 148-L156.
Elias, H., D.M, Hyde (1980): Elementary stereology Am J Anat 159 : 411-446.
Elias, H., D.M Hyde, R. L Scheaffer (1983): A guide to practical stereology Am J Anat 159 :500-580.
Framcos T.F *et al* (1973) Life depends on the liver 1: 50-57.
Gannon M.C *et al* (1997) Diabetologic 40 (70) 758-763.

Gundersen, H.I.G., E.B. Jesen (1987): The efficiency of systematic sampling in stereology and its prediction J.Microse 147:229-263.

Gundersen, H.J.G., T.F.Bentsen. L.Korbo, N. Marcussen, A. Moller.K. Nielsen. J.R. Nyengaard, B. Pakkenberg. F.B Sorensen. A. Veterby, M.J West (1988): Some new, simple and efficient stereological methods and their use pathological research and diagnosis APMIS 96:379-394.

Hollingdaje, M.R. (1985): Malaria and the liver. Hepatology 5:327 -335.

Koranda F.C (1981) : Antimalaria. Journal of the American Academy of Dermatology 4 (6) :650-655.

Loty *et al* (1982) Comparison of the effects of chronic chloroquine treatment and derivation on noradrenergic mechanisms J Med Bio L60 (1) :23 -25.

Machiney J.L.S *et al* (1981) Malaria breakthroughs and resistance to chloroquine in Africa Afr Med J 60 (20) 786-788.

Patricia A Dennis, Nathan N., Aronson J.R. (1981) Effects of low temperature and chloroquine on $I^{125}$ insulin degradation by the perfused rat liver . Archives of Biochemistry and Biophysics 212:170-176.