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The effects of chloroquine on the stereology and histology of the heart muscle of Sprague dawley rats.

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ABSTRACT

Ten male rats were exposed to chloroquine phosphate injection intraperitoneally /IP) for three days. The treated rats received 0.125ml/100g body weight of chloroquine phosphate injection intraperitoneally. Control rats received the same amount of normal saline intraperitoneally.
Histologically, chloroquine caused defects in the structures such as blood vessels. They were constrictions and blood vessels in treated rats compared with controls.
Stereologically, there was a reduction in the estimated absolute volume of treated rats heart muscle compared with controls.
Keywords: Heart muscle, chloroquine,Sprague Dawley rats, stereology, histology.

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, Nivaquine, 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. The exact mechanisms of the 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 Plasmodiam. 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.

MATERIALS AND METHODS

The 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 the normal rat feed from Pfizer PLC Ikeja Lagos. Weight of animals was taken twice daily throughout the duration of the experiment. Ten female rats were 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 intrapertoneally. 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 the animals were sacrificed by diethyl ether decapitation and the rats heart was removed for morphological and histological assessment.

Histological Analysis

The twenty male rats were sacrificed as discussed earlier after treatment with the chloroquine phosphate injection .The rats heart was removed and fixed in Bouin's fluid. The 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 tissue was sectioned using tissues preparation tissues 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 rats heart was made at a final print magnification of 100 and 400 respectively.

5 slides will be obtained from the control and 5 slides from the treated rats.

For each of the fractions, the N_T/A □ number of test points counted on rats heart muscle, per unit area of the fractions was 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 of intestinal glands was 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_T/A (structure).

d= diameter of test grid

M=magnification of projection

The relevant volume densities of the rats heart muscle 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_T/A(structure)

Vv = volume density Nv_R = numerical density ratio

Finally, the absolute volume of rats heart muscle was estimated using this equation.

V(structure) =Vv (structure) x V (ref)

V(structure) = Absolute volumes of structure

Vv(ref) = Reference volume of structure

Statistics
Statistical analysis was carried out using t- Distribution (t- test).

RESULT

TABLE1: MEAN NUMBER OT TEST POINTS PER UNIT AREA (N_T/A) OF HEART MUSCLE

GROUP n=20	Mean (N_T/A)
CONTROL (CO)	140.5
CHLOROQUINE TREATED (CQ)	137.5

CO=CONTROL RATS

CQ= CHLOROQUINE TREATED RATS

TABLE2: MEAN ESTIMATED ABSOLUTE VOLUMES (CM^3) OF HEART MUSCLE

TISSUE	CONTROL RATS n=10	CHLOROQUINE TREATED RATS n=10
HEART MUSCLE	0.36 ± 0.86^a	0.34 ± 1.02^b

a=Mean±S.E.M

b=$p<0.05$

DISCUSSION

Histologically, chloroquine caused defects in the structures of the heart muscle such as,blood vessels.There was constriction in these structures in the treated rats compared with controls.

Stereologically, there was a reduction in the absolute volume of heart muscle in the treated rats compared with controls.

CONCLUSION

Chloroquine should be taken under the supervision of a licensed medical professional

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