



Histological and stereological analysis of the effects of chloroquine on the small intestine of Sprague Dawley rats.

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ABSTRACT

Ten male rats were exposed to chloroquine phosphate injection intraperitoneally/IP) for three days. They were histologically and stereologically investigated. The histology micrograph at a magnification of 100 shows that chloroquine causes defects in the structures of the small intestine. The epithelium was constricted and the number of nuclei in the epithelium reduces in the treated rats compared with controls.

Stereologically, the estimated absolute volume of the lining epithelium of the treated rats small intestine were reduced compared with controls.

Keywords: small intestine, Sprague Dawley rats, histology, stereology, lining epithelium

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) $C_{18}H_{26}ClN_3$ 7- Chloro -4- (4'- diethylamino-1'-methylamino) 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 haemoglobin, 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 ferriprotophyrin IX (FP) which inhibits sequestration of FP into malaria pigment. This could impair haemoglobin 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 desethylchloroquine is also active against chloroquine-sensitive Plasmodium. 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 hydroxychloroquine 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 chemoprophylaxis 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 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 the animals were sacrificed by diethyl ether decapitation and the small intestine 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 small intestine 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 Microtome 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 small intestine 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, per unit area of intestinal villus 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 intestinal villus 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 $V(\text{ref}) = (\text{stratum length}) \times \frac{d^2}{M^2} \times \text{mean } N_T/A (\text{structure}).$

$$M^2$$

d= diameter of test grid

M=magnification of projection

The relevant volume density of intestinal villus of the fractions V_v (structure) was 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:

$$\text{Estimated } V_v(\text{structure}) = N_{vR} \times N_T/A(\text{structure})$$

V_v = volume density N_{vR} = numerical density ratio

Finally, the absolute volume of intestinal villus was estimated using this equation.

$$V(\text{structure}) = V_v(\text{structure}) \times V(\text{ref})$$

$V(\text{structure})$ = Absolute volumes of structure

$V_v(\text{ref})$ = Reference volume of structure

Statistics

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

RESULTS

TABLE1: MEAN NUMBER OF TEST POINTS PER UNIT AREA (N_T/A) OF INTESTINAL VILLUS

GROUP n=20	Mean (N_T/A)
CONTROL (CO)	94.5
CHLOROQUINE TREATED (CQ)	13.5

CO=CONTROL RATS

CQ= CHLOROQUINE TREATED RATS

TABLE2: MEAN ESTIMATED ABSOLUTE VOLUMES (CM^3) OF INTESTINAL VILLUS

TISSUE	CONTROL RATS n=10	CHLOROQUINE TREATED RATS n=10
Small intestine (intestinal villus)	$6.2 \times 10^{-2} \pm 0.86^a$	$8.3 \times 10^{-5} \pm 0.71^b$

a=Mean±S.E.M

b=p<0.05

DISCUSSION

Histologically, chloroquine caused defects in the structure of the small intestine. The intestinal villi were compressed together with very tiny epithelium with few nuclei.

Stereologically, chloroquine reduced the estimated absolute volume of treated rats intestinal villi compared with controls.

CONCLUSION

The small intestine is very vital in the body in digestion and absorption of food in the system. Chloroquine and thus this routine malaria drugs should be taken only if it is prescribed by a licensed medical practitioner.

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