J. Expt. & Clin. Anat. 4(1): 50-54 (2005).

JECA

Effects Of Chloroquine On Some Visceral Organs In The Rabbit: Histopathological Perspective

*A.A NGOKERE[1], T.C. NGOKERE[2], AND O.O. OKECHI[3],
[1]Departments of Morbid Anatomy, [2]Medicine, University of Nigeria Teaching Hospital, Enugu
[3]Surgical Pathology, Abia State University, Uturu, Okigwe - Nigeria
*Author for correspondence

ABSTRACT

The histopathological changes caused by chloroquine phosphate in doses 5, 10, and 15mg/kg over 30, 60 and 90 days in the albino (n = 10) and pigmented (n = 22) rabbits, with mean weight value of 1.40 ± 0.44kg and mean age value of 9. 0 ± 0.25 months were investigated in the college of Medicine, University of Nigeria, Enugu Campus. Histopathology results revealed linear, granular, lumpy and bumpy stainable iron deposition on the wall of the myocardium. The lymphoid organs showed prominent necrosis, fibrosis and disappearance of lymhocytes. Histology of the rabbits' duodenum also revealed marked mucosal ulceration and loss of villi in all treated groups. Patients of malariasis are advised to consult their physicians for drug prescription and proper guidance in chloroquine consumption to avert the toxic consequences of frequent and indiscriminate chloroquine ingestion.

Key Words: Heart, duodenum, lymphoid organ, ulceration, necrosis, histology.

In areas where malaria is endemic and especially in the African continent, Chloroquine has been the mainstay of therapy for malaria (Olatunde, 1977). However, there are many people in this environment who consume chloroquine daily or weekly for months or even years in an attempt to suppress the disease and some of these cases have been documented (Di Maio & Henry, 1974; Edwards et al. 1978). In addition cases abound in the literature of long-term administration of chloroquine, especially in the treatment of rheumatoid arthritis (Hughes et al. 1974, Mangnussen Olivarius, 1977).

Cardiovascular effects resulting from chloroquine administration have been reported in the literature. Parenteral administration of chloroquine in therapeutic doses has been shown to cause a moderate but short lived fall of blood pressure in children (Olatunde, 1970). Similarly, long term administration of therapeutic doses can result in changes in the cardiovascular system, which includes congestive cardiac failure and cardiomyopathy, characterized by vacuolar changes in the myocardium (Hughes et al. 1971; Magnussen & Olivarius, 1977). Acute ingestion of toxic doses either accidentally or in suicidal attempts in humans, or parenteral administration of toxic doses in experimental animals can also cause hypotension and cardiac arrest (Don Michael & Aiwazzadeh, 1970).

In view of the fact that chloroquine is one of the most commonly prescribed drugs and also because many people in malarious environments ingest chloroquine almost daily, it is therefore important to find out the effects of chronic chloroquine administration especially as it affects the heart, duodenum and lymphnodes. Toxic doses were administered parenterally in order to simulate the probable consequences of chronic administration of the drug.

MATERIALS AND METHODS

Animals

Thirty two gnotobiotically reared rabbits each approximately 9 months of age, 22 pigmented and 10 albino were bought from college of Agriculture Isiagu, Enugu State and kept in the animal house of the college of Medicine, University of Nigeria, Enugu campus for 4 weeks for acclimatization. They were housed in bottom wired stainless metal cage and were allowed food and water ad libitum. Individual identification of the animals was by ear tags.

Experimental Design:

The experimental animals were divided into 4 groups comprising 8 sex matched rabbits in each of the 4 groups. Those in groups 1,2 and 3 constituted

the test groups, while the 4th group acted as the control. The animals in group 1 were given doses of chloroquine 5mg/kg/day. The animals in groups 2 received 10mg/kg/day (twice the dose), while the 3rd group received 15mg/kg/day (thrice the dose), all given intraperitoneally doses daily for a period of 90 days. The 4th group received equal volume of normal saline daily.

Experimental Procedure

Two animals from each of the test groups and two from the control group were painlessly sacrificed after 30, 60, and 90 days. All the rabbits were still living when the study terminated and were anaesthetized by intraperitoneal injection of 50mg/kg of sodium thiopentane and were exsanguinated. Necropsies were performed immediately. Representative samples of the hearts, duodenum and lymphnodes were fixed promptly in 10% neutral formal saline, histologically processed using the Elliot automatic tissue processor, embedded in paraffin wax, sectioned at 5μm using Rotary Microtome and stained by Haematoxylin and Eosin technique (Ehrlich, 1886). Two longitudinal and one transverse section of the myocardium were studied in each rabbit.

The Pfizer Pharmaceutical Company Ltd. Lagos, Nigeria supplied the drugs used in this work.

RESULT

Clinical Observation

Clinical changes were observed in the animals receiving treatment with chloroquine. The most consistent clinical findings were sluggishness, depressed appetite, especially in group 3 and poor general appearance. Group 1 receive chloroquine (CHQ) phosphate in the amount of 5mg/kg with no apparent ill effect. Two animals in this group lost weight after the first 2 weeks of the drug administration. Their weights decreased slightly thereafter till sacrifice. The rest of the animals also maintained consistent slight weight loss till sacrifice. Overall, the animals in the control groups had slight weight gains during the experimental duration. On the first day of CHQ administration, one gravid rabbit in group 2 aborted spontaneously 3 hours later, whereas on the 9th day of treatment, 2 of 8 animals aborted in group 3.

The obvious clinical changes in the rabbits in the test groups were mild dermatitis, which affected more of the albino rabbits than the pigmented and faecal hyperpigmentation. Onset of dermatitis was at day 65 and involved the entire body, which was found dry and scurfy with prominent loss of hair in most albinos and in some of the pigmented on which bleaching of hair was also observed. All the rabbits in all the groups survived the treatment regimen and generally experienced slight weight losses throughout the test period. The rabbits in the control group appeared in excellent health condition throughout the period of the study.

Gross Anatomy and Pathology:

The heart, duodenum and the lymph nodes showed normal colour, size and consistency in the control group, whereas the gross pathology of these organs in groups 1, 2 and 3 showed some changes. Multiple haemorohages were seen in the heart, lungs and stomach. The lymphnodes appeared slightly inflamed. These observations were especially noted in the animals in groups 2 and 3 particularly. Alopecia, dermatitis and hyperpigmentation of faeces due to stomach haemorrhage occurred in the rabbits in all the test groups. Bullae were also observed on the abdominal regions of the animals in all the study groups, particularly 2 and 3. After some time the bullae were observed to have ruptured and the site ulcrated, the ulcers healed with scar tissue formation.

Histological Examination

Light microscopic examination of the H & E stained sections of the hearts of rabbits on chloroquine in groups 1 & 2 therapy did not show any evidence of vacuolar or other changes in the myocardium. However, the myocardium of the rabbits in group 3 showed linear, granular, bumpy and slumpy deposition of iron when stained by perls method (Perls, 1867) which is suggestive of degenerative changes (Fig1). Areas of focal or confluent fibrinoid necrosis of individual myocardial fibres were not seen even at high magnification (x 1000). The myocardia of those in the control group showed normal architecture (Fig 2) Fig 3 represents the photomicrograph of the duodenum displaying features of mucosal denudation burrowing into the Brunner's glands. Fig 4 represents the same duodenum showing loss of villi in all the treatment groups while Fig 5 showed duodenum with normal architecture. Fig 6 represents a lymphoid organ (lymphnode) showing piecemeal necrosis and early

fibrosis pervading the stroma. The section of the lymphnode showed paracortical diffuse necrosis and a thick fibrous band separating the necrosed area and the deranged solitary follicle (Fig 7) in the treated groups Fig 8 is a section of lymphoid organ showing prominent diffuse necrosis in the tissue stroma resulting in loss of lymphocytes in 15mg/kg dose group. Fig 9 represents the photomicrograph of lymphnode section with normal architecture from the control group. All the histologic lesions varied in intensity in the various treatment groups and were all dose dependent. In all cases there was disappearance of germinal centres in lymphoid follicles.

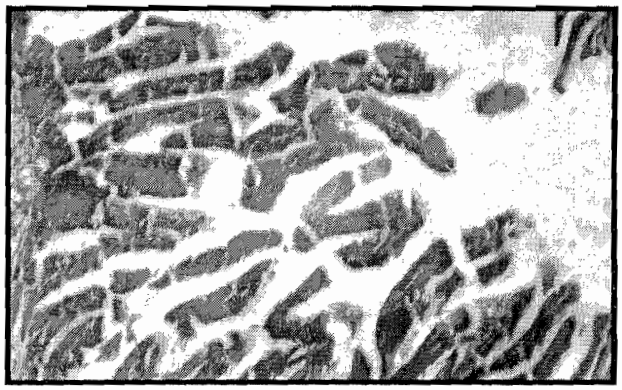

Fig 4 is a photomicrograph of the duodenum from all the treated groups displaying loss of villi stained by H & E. X 100

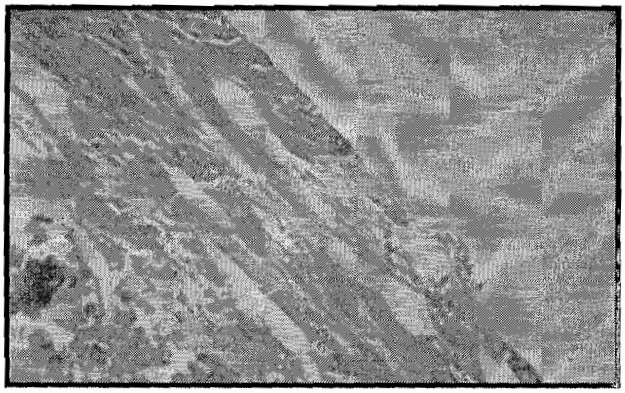

Fig 1 represents the photomicrograph of myocardium of the heart showing almost a linear, granular, bumpy and lumpy stainable iron deposition on the wall. Stained by H & E Technique. X 100.

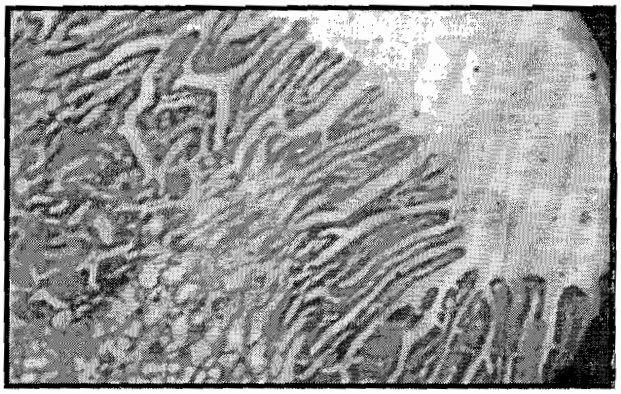

Fig 5 is a cross section of duodenum from the control rabbit showing normal archilecture.

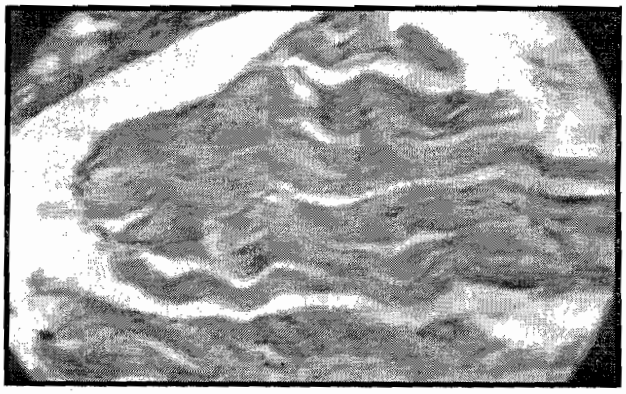

Fig 2 shows myocardium with normal archictecture from the control rabbit heart.

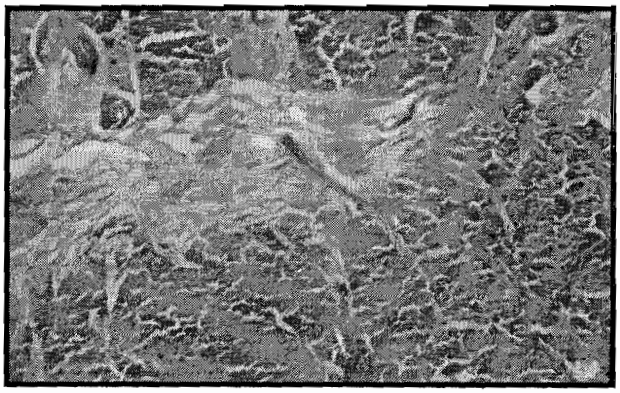

Fig 6 represents the photomicrograph of lymphnode of rabbit in group 1 at day 30 showing necrosis and fibrosis pervading the tissue stroma. Stained by H & E. X 100

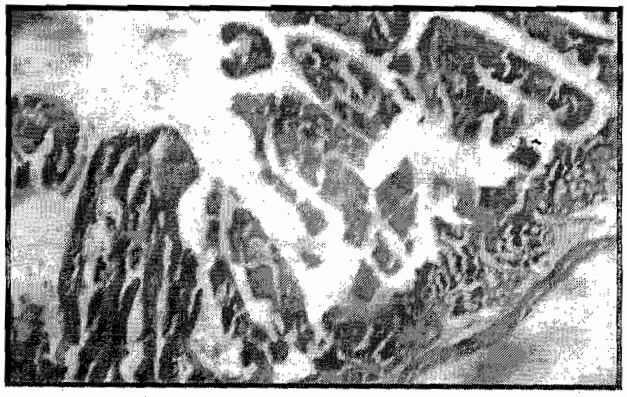

Fig 3 represents the photonicrograph of duodenum of the treated rabbit showing mucosal ulceration. The ulceration has encroached the Brunner's glands stained by H & E Technique. X 100

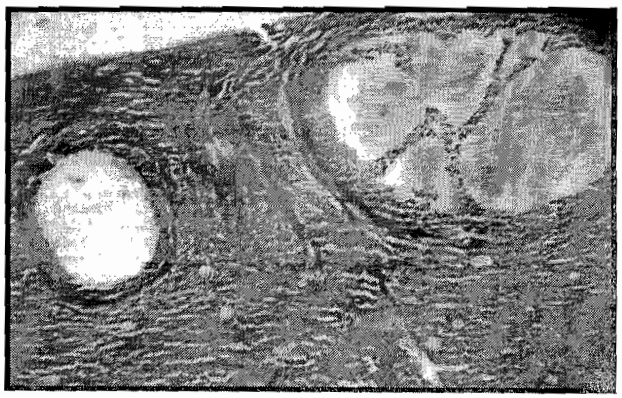

Fig 7 shows prominent diffuse necrosed area separated from deranged solitary follicle displaying lymphocyte loss by a band of fibrous tissue. Stained by H & E technique. X 100

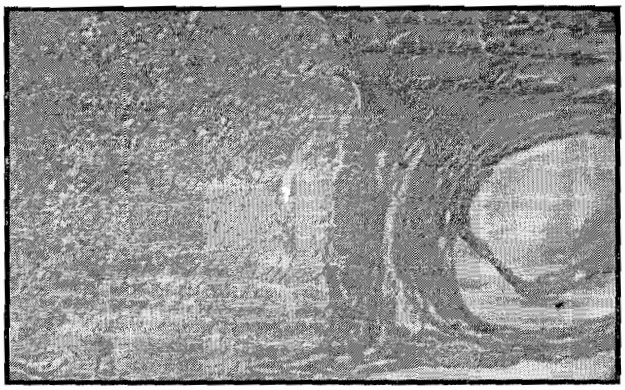

Fig 8 seprenents a cross section of lymphoid organ showing diffuse prominent necrosis. Prominent lymphocyic loss is obvious in the tissue stroma. Stained by H & E technique. X 100

Fig 9 show a lymphnode from the rabbit in the control group displaying Normal architecture. Stained by H & E technique. X 100.

DISCUSSION

Histological examination in this present series revealed the presence or deposition of linear, granular, lumpy and bumpy stainable iron on the wall of the myocardium. This finding is suggestive of degeneration of the myocardium. Our failure to find myofibril and necrosis of the muscle fibres is in contrast to the observation that rabbits given an equivalent amount of chloroquine develop vacuolar myopathy in the heart, usually after 2 weeks of drug administration (smith O' Grady, 1966). The negative vacuolar myopathic changes in the present series may be due to species variation in the resistance of the heart to damage. However, this is not completely true because it has been demonstrated that, in rats, administration of CHQ (30 40mg/kg) for 2-4 weeks results in changes in myofibrils and mitochondria of the heart on electron microscopy (Ayitey Smith & Gbewonyo, 1977). Our findings therefore may reflect the possibility that ultrastrctural changes may have been occurring in the heart, which were not detectable by our technique of light microscopy. The electron microscopic evidence in rats and the light microscopic evidence in rabbits, already mentioned, provide additional evidence that chronic CHQ administration, by damaging the contractile elements of the heart, causes myocadiac depression which may have contributed to the hypotension observed in a separate study (Sofola et al. 1981). Thus chronic administration of toxic doses of CHQ, which may simulate long term administration in man, results in a persistent hypotensive effect in rats, which is reversible on withdrawal of the drug. The duodenum showed mucosal denudation, which encroached into the Brunner's glands. Loss of Villi chronic following CHQ administration was also noted in the present series. Both the stomach and duodenal ulceration in the rabbit could have been responsible for the faecal hyperpigmentation due to haemorhage as noted in our clinical observation of the animals. Duodenal ulceration induced by chronic administration of CHQ, to the best of our knowledge, has not been documented in literature previously. The effect of CHQ administration on lymphnodes consists of degeneration of lymphocytes and reduction of lymphoid cells due to necrosis as obtained in our series. We observed that the effect of CHQ on the lymphnode include necrosis, lymphoid atrophy, lymphocytic loss and stromal fibrosis. (cope, 1972; katzung, 1996). It has also been observed that surviving lymphoid cells show evidence of degeneration and severe degree of evolutionary changes in all lymphnodes of the body without seriously interfering with the functional activity of the lymphoid tissues which survive (cope, 1972; Memmier and Wood, 1972). The nuclear and cytoplasmic materials from the destroyed lymphocytes are phagocytosed by the fixed reticulo endothelial cells. The destruction of the lymphocytes was clearly shown by the disappearance of the nuclear and cytoplasmic staining reaction product which may be due to atrophy of the lymphocytes or movement of the lymphocytes out of the nodes to the general circulation (cope, 1972; Ibegbu et al, 2001). It has been demonstrated that mitosis of the lymphocytes are also inhibited and both reduced formation and greater reduction in lymphocyte population contribute to lymphocytopenia (cope, 1972). The necrosis in the lymphoid organs may lead to reduction in number of lymphocytes following degeneration and necrosis may contribute to reduction in antibodies forming cells. This may as well contribute to the reduction in resistance of the rabbits to infection, thus causing immunosuppression in the animals exposed to chromic CHQ treatment.

Patients of malariasis are advised to consult their doctors for drug prescription and proper guidance in chloroquine ingestion to avert the toxic consequences of frequent and indiscriminate chloroquine consumption.

Table1: Dosage effects of chloroquine on rabbit organs

Groups	Chloroquine Dose	Chemical	Gross Pathology	Histologica (General)
Group 1	**5 mg/kg /day**	**Loss of weight in 2 animals**	**Hacemorrhage in the internal organs, bullae, Damatitis**	**Necrosis and fibrosis of lymphnode Duodenal ulcer and loss of villi. No myopathic changes in groups 1 and 2**
Group 2	**10 mg/kg/day**	**Abortion in one animal**	**Haemorhages, bullae, dermatitis**	
Group 3	**15 mg/kg/day**	**2 animals aborted on the 9th day**	**Slightly shrunken nodes, inflamed duodenum, haemorrhages, - bullae – dermatitis**	**Linear, granular, slumpy and bumpy iron deposit on the myocardial wall, duodenal demudation villi loss, Prominent fibrosis, necrosis and loss of lymphocytes in the nodes**
Group 4 control General	**Equal volume of normal saline - dermatitis - alopecia**	**- excellent good health**		

ACKNOWLEDGEMENT

We thankfully acknowledge the entire staff of the Animal house Department of the College of Medicine, U.N.E.C. We also appreciate the effort, advice and guidance of DR. A.A. Ofodile in the preparation of this manuscript. We are also indebted to Augustine Okoye for his technical assistance. The secretarial assistance from Dan Ugwu is a welcome contribution.

REFERENCES

Ayitey Smith E; and Gbewonyo A.J.K (1977) ultrastructure changes in the heart of rats treated with chloroquine. West Afr. J. Pharmacol. And Drug Res **4**;

Cope L.G (1972). Adrenal steroids and Diseas Pitman. Company, New York Pp. 235 -553

Di Maio V.J.M and Henry L.D (1974). Chloroquine posoning. South Afr. Med. J **67**; 1031

Don Michael T.A; and Aiwazzadeh S (1970) The effect of acute chloroquine poisoning with special reference to the heart. Am. Heart .J 79;831

Edwards A.C; Meredith J.J; and Sowton, E (1978). Complete heart block due to chronic chloroquine toxicity managed with permanent pacemaker B.M.J n; 1109

Erhlich, P (1886). Demonstration of tissue structure .Z. Wiss Mikr; **3**:150

Hughes J.T; Esiri M; Oxbury J.M; and Whitty C.W.m (1971). Chloroquine myopathy. Quarterly Jounal of Medicine **40**:85

Ibegbu A.O; Singh S.P; Aliyu A.A; Shuaiba O.I; and Ezemagu U.K (2001). The effect of steroids on some Haematological indices of Albino Rats. J. Vat. Res. **54**:101-135

Katzung B (1996): clinical pharmacology, Lange medial publi California Pp 135

Magnussen 1; and Olivarius B. de F (1977). Cadiomyopathy after chloroquine treatment. Acta medica scandinavica **202**: 429

Smith B; and O' Grady F (1966) Experimental Chloroquine myopathy. J. Neurology and Neurosurg. **29** : 255

Sofola O.A; Olude I.O and Adegoke F (1981). The effect of chloroquine toxicity on blood pressure of rats. J. trop. Med. & hyg. **84**:249 252

Olatunde I.A (1977). The practical and therapentic implications of chloroquine induced itching in tropical Africa African Journal of Medical Science **6**:27

Olatunde I.A (1970). Parenteral chlorquine in children. West African Medical Journal **19**:93

Perls, N (1867). Demonstration of ferric iron. Virchows Arch. Path Anat; **39**:42