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Nephrotoxicity Of Chloroquine In The Rabbit

A.A NGOKERE[1*] AND T.C. NGOKERE[2]
Departments Of Morbid Anatomy[1] And Medicine[2],
University Of Nigeria Teaching Hospital, Enugu, Nigeria.

ABSTRACT

The histopathologic and biochemical changes produced 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 1.46 ± 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 showed that chloroquine induced lesions in the kidney. Peritubular and interstitial mononuclear cell infiltration, marked tubular dilation, edema, necrosis and hyalinization as well as glomerular degeneration and loss in some instances were observed. The electrolytes were significantly ($P < 0.05$) found retained except the bicarbonate ions, which were significantly ($P < 0.05$) decreased at different periods of the study. Both findings reflect renal function impairment and lowering of G F R. Chloroquine should be taken with caution in view of its toxic potential.

Keywords: Kidney, Histology, Renal function, Electrolytes, Ketoacidosis.

Chloroquine has been widely used in man for the treatment and suppression of malaria and for prolonged treatment of chronic diseases such as rheumatoid arthritis, discoid and systemic lupus erythematosis (Seideman et al, 1994). Malaria continues to represent a major public problem in tropical countries in terms of geographical spread, high mortality and severe morbidity, especially among children. Two billion people, or about 40% of the world's population, live in the 90 countries at risk (WHO, 1994). Global estimates of the malaria diseased burden for 1992 indicated at least 300 500 million clinical cases were recorded annually, 90% of them in sub- Saharan Africa. Chloroquine is one of a large series of 4 aminoquinolines investigated as part of the extensive cooperative program of antimalarial Research in the United States during World War II. The objective was to discover more effective and less toxic suppressive agents than quinacrine. Although the 4 aminoquinolines had been described by Russian investigators, serious attention was not paid to this chemical class until the French reported that one derivative, Sontochin, was well tolerated and had high activity in human malarias.

Beginning in 1943, thousands of these compounds were synthesized and tested for activity. Chloroquine eventually proved most promising and was released for field trial. When hostilities caused, it was discovered that the chemical had been synthesized and studied under the name of Resorchin as early as 1943 by the Germans (Webster, 1996). Prolonged treatment of chloroquine may cause lichenoid skin eruption in a few patients; the condition is mild and subsides promptly when the drug is discontinued. Readministration of chloroquine (CHQ) does not result in the reappearance of the lesion. Large doses given for a year to a group of healthy volunteers occasionally caused some visual symptoms, bleaching of the hair, T- wave abnormalities, and headache and were reversible upon withdrawal of the drug (Aluing et al, 1948). High daily doses (>250mg) of chloroquine, used for long-term treatment of diseases other than malaria, can result in irreversible retinopathy. This complication is presumably related to deposition of drug in melanin rich tissues (Berntein et al, 1963). Prolonged daily therapy with high doses of chloroquine has been known to cause toxic myopathy, cardiomyopathy, and peripheral neuropathy; these reactions are reversible if the drug is discontinued promptly (Estes et al, 1987).

It is not known whether the present high incidence of nephropathy with its associated high mortality rate in our society has any relationship with increased chloroquine consumption. In this particular case, it is important to know whether chloroquine consumption is implicated in nephropathy and to create awareness among the masses particularly the villagers in the rural areas and the uninformed. The current study was intended to determine the clinical, observation, biochemical, gross and light microscopic changes induced by chloroquine (CHQ) in the kidney

of rabbits, and to ascertain the onset of toxicity and its relationship with dose as well as the pattern of progression of chloroquine induced histologic lesions with time.

MATERIALS AND METHODS

Animals.

Thirty-two gnotobiotically reared rabbits each approximately 9 months of age, 22 pigmented and 10 albino were procured from the Veterinary Research Institute, Vom, Nigeria 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 metal cages 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 group 2 received 10mg/kg (twice the dose) while the 3rd group received 15mg/kg/day (thrice the dose), all given intraperitoneally dose daily for a period of 90 days. The 4th group received equal volume of normal saline daily.

Experimental Procedure:

Blood samples were taken from the marginal ear for biochemical parameters prior to drug administration. Thereafter, similar samples were collected at day 30, 60 and 90. Blood samples collected prior to drug administration prôduced biochemical values, which represented the baseline values. 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 intraperitonal injection of 50mg/kg of sodium thiopentane and were exsanguinated. Necropsies were performed immediately. Representative samples of the kidney were fixed promptly in 10% neutral formol saline, histologically processed using the Elliot automatic tissue processor, embedded in paraffin wax, sectioned at 5ì thick using the Rotary microtome and stained by Haematoxylin and Eosin (H&E) technique (Ehrlich, 1886).

The Pfizer pharmaceutical company Ltd. Lagos, Nigeria supplied the drugs used in this work.

Statistical Analysis:

The results were expressed as Mean ± SEM and significance of differences between control and treated as well as before and following treatment for biochemical parameters were determined using paired student t- test and one-way analysis of variance (ANOVA). Statistical significance were set at $P < 0.05$.

Samples Collection:

Whole blood samples for serum electrolytes were collected in plain test tubes.

Biochemical Analysis:

The methods used for the estimation of sodium, potassium, chloride and bicarbonate electrolytes were according to Tietz (Tietz, 1970), Schales and Schales (Schales and Schales, 1941) and Van Slyke (Van Slyke, 1969) respectively.

RESULTS

Clinical Observation:

Clinical changes were observed in the animals receiving chloroquine phosphate. The most consistent clinical findings were sluggishness, depressed appetite, especially in-group 3, and poor general appearance. Group 1 received chloroquine 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. The rest of the animals in the test groups also maintained consistent slight weight loss till sacrifice. On the first day of CHQ administration, one gravid rabbit in group 2 aborted spontaneously one fetus 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 hyperpigmentation of the faces. Onset of dermatitis was at day 65 and involved the entire body, which was found dry and scurfy with prominent hair loss 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 gains and some losses throughout the treatment period. The rabbits in the control group appeared in excellent health condition throughout the period of study.

Gross-anatomical observation:

The kidneys obtained from the control group showed no differences in their normal gross anatomical features, i.e. Size, colour and consistency.

Alopecia, dermatitis and lyperpigmentation occurred in the rabbits in all the test groups. Bullae were observed on the abdominal regions of the animals in all the study groups, particularly 2 and 3. After sometime the bullae were observed to have ruptured and the sites ulcerated. Thereafter, the ulcers healed with scar tissue formation. At necropsy, kidney showed cortical depressions and slight shrunkenness in some instances.

Histologic findings:

The most significant lesions observed in the kidney of the rabbits in the treatment groups were degeneration and necrosis of the tubular epithelium. Degeneration was observed as a marked vacuolation of the cytoplasm of the tubular epithelial cells. At 5mg/kg dose of CHQ at day 30, mild tubular dilation necrosis and medullary rays hyalinization and mixed inflammatory cell infiltration of the stroma were observed. At 10mg/kg dose, the nephrons showed glomerular loss and degeneration as well as hyaline droplets in the tubules. (Fig1) Tubular dilatation and necrosis were also evident. Paracortical chronic inflammatory cell infiltration of the interstitium, tubular droplet hyalinization and degeneration were noted in both 10 and 15mg/kg dose groups at day 60. (Fig. 2) Kidney sections of rabbits in the 15mg/kg dose category displayed marked tubular hypertrophy, necrosis, peritubular mononuclear cell infiltration and glomerular degeneration at day 90. (Fig.3) Prominent tubular dilation, edema and necrosis were noted in 1 of 6 sacrificed animals at 15mg/kg dose at day 90. (Fig.4). Glomerular degeneration, pyknosis, hyperchromasia and shrunkenness were also observed in the section of the same animal. Following this pathology, renal urinary spaces were obviously wider. In this case, miminal enlargement of the Bowman's capsule was noted. Haemorrhage and early fibrosis was also evident. (Fig.5) Kidney sections from the control group showed normal architecture. (Fig. 6) In all instances, necrosis and edema were seen as excessive eosinophilia of the epithelial cytoplasm and pyknosis of the nuclei of these cells. These changes were patchy or focal and of mild and severe intensity. In a few other cases, haemorrhage as well as early fibrosis was evident.

Inflammatory cell infiltration was suggestive of interstitial nephritis. These collective observations suggest that acute and chronic doses of CHQ induced renal lesions had been produced and indicate renal failure.

Biochemical analysis:

The electrolyte profile in these rabbits were determined and evaluated to ascertain the functional integrity of the renal tubules.

Table 1 shows the mean (mean $\pm$ SEM) values in mmol/L of serum sodium (Na^+) electrolyte. The baseline and test mean values were 134.6 $\pm$ 1.49 and 142.0 $\pm$ 1.31, 144.45 $\pm$ 1.57 and 145.0 $\pm$ 1.58 at days 30, 60 and 90 respectively. The increases in test mean values over the baseline were statistically significant ($P<0.05$). This result has shown that sodium electrolyte level in serum was significantly elevated during the periods of study and that the increases following CHQ administration was only dose and not time dependent in group 1. In group 2, the baseline and test mean values were 1370 $\pm$ 0.38, and 139.9 $\pm$ 0.85, 141.71 $\pm$ 0.80 and 143.0 $\pm$ 1.29 at days 30, 60 and 90 respectively. Although there were statistically significant ($P<0.05$) increases in test serum sodium electrolyte over the baseline the ANOVA was not statistically significant $P>0.05$. These results showed that CHQ caused very significant increases in the retention of sodium electrolyte and which increases were dose dependent. In group 3, the baseline and test mean values were 130.91 $\pm$ 1.46 and 136.9 $\pm$ 0.58, 139.5 $\pm$ 0.99 and 145.5 $\pm$ 2.26 mmol/L over the period of study. The increases of test mean values over the baseline were statistically significant ($P<0.05$ at the different periods of study. In this case, CHQ caused increased Na^+ retention in circulation of the rabbits.

Table 2 displays the pattern of potassium (K^+) electrolyte distribution in blood sera of the rabbits. The baseline and test mean values in group 1 were 4.25 $\pm$ 0.30 and 4.55 $\pm$ 0. 24, 4.80 $\pm$ 0.38 and 4.90 $\pm$ 0.27 at days 30, 60, and 90 respectively. Although the tests showed numerical increases in values over the baseline, these increases were not statistically significant ($P>0.05$) . In this group CHQ -induced increases were neither dose nor time dependent. Group 2 showed that the baseline and tests produced mean values of 4.2 $\pm$ 0. 28 and 4.5 $\pm$ 0.26, 4.93 $\pm$ 0.30 and 5.5 $\pm$ 0.43 mmol/L. It was observed that there were slight increases in test mean values over the baseline; they were not statistically significant ($P>0.05$). The result has shown that there were slight increases in numerical values of serum potassium electrolyte.

Chloroquine at 10mg/kg dose did not cause statistically significant increase in serum potassium (k^+) level. On the contrary, the 15mg/kg dose produced the baseline and test mean values of 4.30 ± 0.12 and 4.93 ± 0.32, 5.0 ± 0.24 and 5.08 ± 0.33 over the period of estimation. However, the test mean values increased significantly ($P<0.05$) over the baseline Overall, the results had shown that in group 3 CHQ caused increased serum K^+ retention and was only dose dependent Table 3 shows serum chloride (CL^-) values at the different periods of study. The baseline and test mean values were 101.5 ± 5.63 and 104.3 ± 4.1, 111.3 ± 3.01 and 117.8 ± 1.89 at days 30, 60 and 90 respectively. The test mean value increases over the baseline were statistically significant ($P<0.05$) at days 60 and 90 respectively and not statistically significant ($P>0.05$) at day 30. The analysis of variance was not significant ($P>0.05$). This result had shown that in group 1, CHQ produced statistically significant increases in the serum CL^- retention from day 60 up to day 90 but was not duration dependent. In group 2, the baseline and test mean values were 102.9 ± 9.58 and 109.5 ± 3.51, 116.5 ± 5.21 and 125.0 ± 5.9 at 30, 60 and 90 days respectively. It was observed that the test mean increases over the baseline were statistically significant ($P<0.05$) over the period of study. The ANOVA was not statistically significant ($P>0.05$). The results showed that CHQ administration in group 2 produced statistically significant retention of CL^- in the rabbits at days 60 and 90 respectively and the increases were dose and not time dependent.

In group 3, the baseline and test mean values were 102.5 ± 3.12 and 107.0 ± 1.85, 108.7 ± 3.01 and 112.5 ± 4.12 at days 30, 60 and 90 respectively. The test mean values showed statistically significant ($P<0.05$) elevations over the baseline. These results showed that the overall statistically significant increased retention of CL^- was both dose and time dependent.

Table 4 displays the values of serum bicarbonate ions at 5, 10 and 15mg/kg dose regimen. The baseline and test mean values were 13.38 ± 0.84 and 21.13 ± 0.88, 17.83 ± 0.95 and 14.25 ± 0.85mmol/L at days 30, 60 and 90 respectively. It was noted that there were statistically significant decreases ($P<0.05$) in the test mean values at different periods of estimation. This result showed that CHQ administration in group 1 caused a very significant decrease in serum BCO_3^- level in the rabbits. In the same vein, group 2 animals showed that the baseline and test mean values were 24.75 ± 1.26 and 21.0 ± 0.87, 18.0 ± 1.16 and 14.0 ± 1.26 at days 30, 60 and 90 respectively. The decreases in test mean values over the baseline were statistically significant ($P<0.05$). The results obtained showed that in group 2 CHQ administration caused statistically significant fall in serum BCO_3^- level in the rabbits over the period of study. In group 3 as well, the baseline and test mean values were 25.0 ± 0.46 and 20.63 ± 0.38, 17.83 ± 0.65 and 13.25 ± 0.48 at days 30, 60 and 90 respectively. The decreases were statistically significant ($P<0.05$) over the period of estimation. The overall decrease was both dose and time dependent.

DISCUSSION

The current investigation has proved that CHQ caused prominent structural changes in the kidney, which could lead to nephropathy as evidenced in patients attending the Renal clinic in our institution. The tubules were observed to show marked dilation and necrosis. Some nephrons showed glomerular loss, glomerular degeneration, pyknosis, shrunkenness and hyperchromasia. Overall, there was haemorrhage as well as interstitial mononuclear cell infiltration coupled with tubular hyalinization. Our finding that CHQ produced edema in the tubules is not far from the work of other authors (Kellner et al, 2000) who made similar finding in the retina. In this series, clinical observation of the animals showed pigmentary changes in the face, which strongly agrees with that of Bentsi-Enchill (Bentsi-Enchill, 1977) who noted similar observation in his patients. Alopecia is a clinically distinct variant of lichen planopilaris that affects in particular elderly women and frequently involves the eyebrows and the basis for lichenoid tissue reaction targeting frontal scalp follicles and eyebrows is unknown (Kossard and Wilkinson, 1997). The finding of alopecia, in our own case, was progressively a frontal fibrosing one. Chloroquine has been known to be abortifacient (Madati, 1971). It is not surprising, therefore, that the experimental animals spontaneously aborted during the course of chloroquine treatment. The inducement of abortion in these animals could not be ascribed to uterine contractions but due to dysmorphogenicity and embryotoxicity of chloroquine effects (Ambroso and Harris, 1993). The effect of 3 consecutive days of oral CHQ (lmg/100g body weight) on kidney function and blood pressure have been studied in male Sprague-Dawley rats that were challenged with hypotonic saline infusion 24h after the last chloroquine administration. These investigators (Musabayane et al, 1994) found that kidney function was compromised in chloroquine-treated rats, which retained significantly more of the infused Na^+ and CL^- by comparison to control-vehicle-treated rats. This observation consonnates well with our finding of significant elevation of both Na^+ and CL^- retention at different periods of the study. At 5, 10, and

15mg/kg dose regimen Na^+ retention was all dose dependent and only dose and time dependent at 15mg/kg dose.

Chloride ion retention was only dose dependent at days 60 and 90 at 15mg/kg dose regimen. At days 60 and 90, the 10mg/kg dosed animals showed dose and not time dependent retention but at 15mg/kg dose the retention became both dose and time dependent. Retention of K^+ was neither dose nor time dependent in 5 and 10mg/kg dose regimen, but showed dose and time dependence at 15mg/kg dose

Our finding that BCo_3^- levels decreased significantly reflects ketoacidosis, whereas the retention of Na^+, CL^- and K^+ at different periods of the study is a clear indication of impairment in renal function and lowering of Glomerular function rate (GFR).

In this part of Nigeria, most patients strongly believe that medical consultation is both time consuming and costly, so they dread going to hospitals in times of malariasis and other ailments and resort to self-medication or quacks to overdose themselves with chloroquine. To them, every pathological condition is due to malaria. The medicare team should discourage the current practice of drug abuse by mounting a comprehensive enlightenment campaign to educate the masses on proper and appropriate use of drugs including CHQ

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TABLE 1: MEAN VALUES OF SODIUM (Na^+) ELECTROLYTE (mmol/L)

Group	Baseline	Day 30	P value	Day 60	P value	Day 90	P value	ANOVA
1	134.6 ± 1.49	142.0 ± 1.31	*P = 0.0023	144.45 ± 1.57	P = 0.0007	145.0± 1.58	P= 0.0015	P= 0.6184
2	137.0 ± 0.38	139.9 ± 0.85	P = 0.0082	141.71 ± 0.80	P < 0.0001	143.0± 1.29	P= 0.0002	P= 0.1838
3	130.9 ± 1.46	136.9 ± 0.58	P = 0.0019	139.5 ± 0.99	P = 0.0007	145.5± 2.26	P= 0.0002	P = 0.0089
4	134.5 ± 0.65	136.0 ± 0.80	-	135.2 ± 1.14	-	135.5± 1.19	-	-

* P< 0.05 considered significant

TABLE 2: MEAN VALUES OF POTASSIUM (K^+) ELECTROLYTE (mmol/L)

Group	Baseline	Day 30	P value	Day 60	P value	Day 90	P value	ANOVA
1	4.25±0.3	4.55±0.24	P=0.4439	4.8±0.38	P=0.2674	4.9±0.27	P=0.1936	P=0.1557
2	4.2±0.28	4.5±0.26	P=0.4329	4.93±0.3	P=0.1069	5.5±0.43	P=0.0879	P=0.234
3	4.3±0.12	4.93±0.22	P=0.0264	5.0±0.24	P=0.0168	5.08±0.33	P=0.0207	P=0.788
4	4.29±0.10	4.33±0.10	-	4.33±0.12	-	4.25±0.13	-	-

* P< 0.05 considered significant

TABLE 3: MEAN VALUES OF CHLORIDE IONS (CL^-) (mmol/L)

Group	Baseline	Day 30	P value	Day 60	P value	Day 90	P value	ANOVA
1	101.5 ± 5.63	104.3 ± 4.1	P = 0.2828	111.3 ± 3.01	P = 0.0023	117.8 ± 1.89	P= 0.003	P= 0.5131
2	102.9 ± 9.8	109.5 ± 3.57	P = 0.087	116.5 ± 5.21	P = 0.0087	125.0 ± 5.9	P= 0.0019	P= 0.0917
3	102.5 ± 3.12	107.0 ± 1.85	P = 0.0055	108.7 ± 3.01	P = 0.003	112.5± 4.12	P= 0.0008	P= 0.456
4	102.6 ± 0.86	102.9 ± 1.20	-	102.5 ± 1.26	-	103. 5± 0.66	-	-

* P< 0.05 Considered Significant

TABLE 4: MEAN VALUES OF BICARBONATE (BCo_3^-) (mmol/L)

Group	Baseline	Day 30	P value	Day 60	P value	Day 90	P value	ANOVA
1	23.38 ± 0.84	21.13 ± 0.88	P = 0.0182	17.83 ± 0.95	P = 0.0002	14.25 ± 0.85	P< 0.0001	P< 0.0001
2	24.75 ± 1.26	21.0 ± 0.87	P = 0.0282	18.0 ± 1.16	P = 0.0025	14.0 ± 0.71	P= 0.0002	P= 0.0003
3	25.0 ± 0.46	20.63 ± 0.38	P < 0.0001	17.83 ± 0.65	P < 0.0001	13.25 ± 0.48	P< 0.0001	P< 0.0001
4	24.75 ± 0.53	24.75 ± 0.41	-	24.5 ± 0.92	-	24.75± 0.48	-	-

* P< 0.05 considered significant

Fig 1 shows that at 10mg/kg dose, the nephrons showed glomerular loss, degeneration as well as hyaline droplets in the tubules.

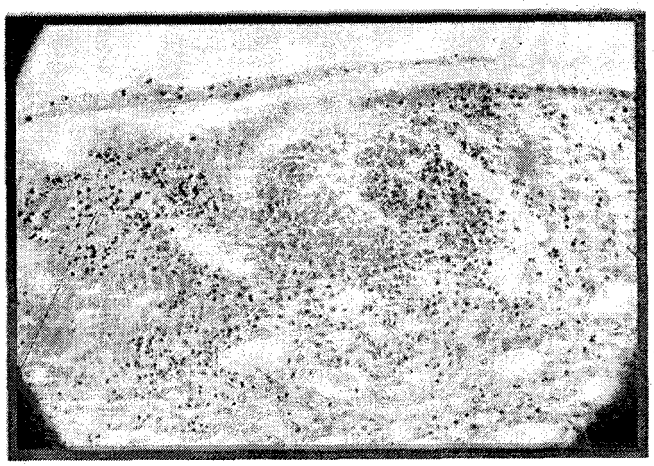

Fig2 displays the paracortical chronic inflammatory cell infiltration of the interstitium and tubular droplet hyalinization and degeneration in both 5, 10 and 15mg/kg due groups at day 60.

Fig.3 shows kidney sections of rabbits in the 15mg/kg dose category displaying marked tubular hypertrophy, necrosis, peritubular mononuclear cell infiltration and glomerular degeneration at day 90.

Fig. 4 shows evidence of prominent tubular dilatation, edema and necrosis in I of 6 treated rabbits at 15mg/kg dose at day 90.

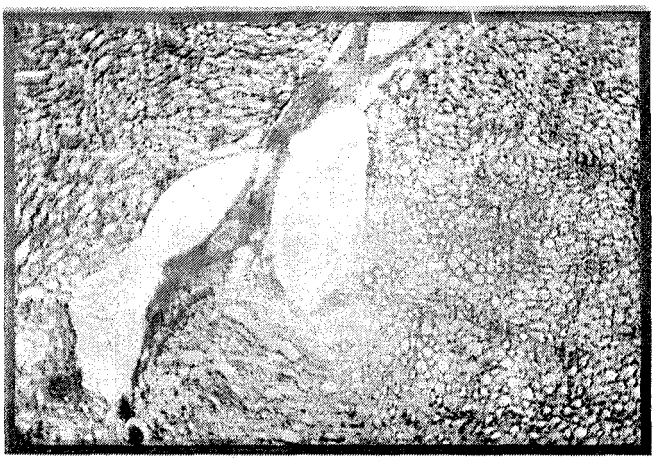

Fig 5 shows evidence of haemorrhage and early fibrosis in the kidney stroma at day 90 of drug administration.

Fig 6 displays normal architecture of the kidney in the control group.

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