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URINARY EXCRETION OF CHLOROQUINE AND ITS METABOLITES

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The amount of individual metabolites of chloroquine present in the urine of Wistar Albino rats was determined. The desethylchloroquine and the 4 - amino - 7 - chloroquinoline were identified in this study similar to those obtained in man by previous investigations. The results of the investigation suggest that rat metabolises chloroquine in a similar way as man.

Key words: Chloroquine and its metabolites, Urinary excretion, Albino rats.

Introduction

Chloroquine, an antimalarial drug which was introduced into therapeutics in 1946 remains the most important drug for the treatment of malaria and its importance has never been seriously challenged. As a result ,several research programmes have been embarked on more understanding of the nature of the drug, particularly the metabolic rate (Mc Chesney *et al* 1966).

Several studies about the metabolism of chloroquine both in animal and man (Titus *et al* 1948; Kuroda 1962; Mc Chesney *et al* 1966; Essien 1978; Adelusi 1982) have been published. However, only the identification of the metabolites was undertaken and no attempt was made to quantify the amount of these metabolites present in body fluid.

The study conducted in the rat for the urinary levels of chloroquine in relation to dietary protein determined the amount of chloroquine and total amount of metabolites in the rat (Adelusi 1982). No attempt was made to identify and quantiy the various metabolites.

The type of metabolites identified in the previous studies include: desethylchloroquine, bisdesethychloroquine, 4' - hydroxy chloroquine and 4-amino-7-chloroquine (Titus *et al* 1948; Kuroda 1962). Recently both the N-oxide and di-N-oxide metabolites of chloroquine have also been identified (Essien 1978).

In the present investigation, the metabolites of chloroquine in the urine of rats using thin layer chromatographic technique have been identified and the amount of the metabolites present is also determined.

Materials and Methods

The experiment was conducted on male Albino rats of wistar strain supplied by the Department of Pharmacology and Toxicology, University of Benin. Chloroquine (CQ) as the phosphate and the metabolites, desethylchloroquine (DQ), bisdesethylchloroquine (BQ) and the 4-amino-7-chloroquinoline were gifted from Walter Reeds, Washington D.C., U.S.A.

Collection of urine from the experimental animals. The rats were weighed and kept in metabolic cages where they were allowed to drink water and have normal feed. They were however allowed to adopt themselves to the environment by leaving them in the cages for two days. After two days, zero hour urine (So) was collected from the animals. which served as a control since no chloroquine was administered to the rats initially.

Chloroquine was administered into the rats intraperitonically at a dose of 10mg kg^{-1} chloroquine base by injecting 1ml of the 2mg ml^{-1} chloroquine phosphate in normal saline.

After the chloroquine administration, urine was collected at the intervals of one, three, five, seven, twenty-eight and forty-two days respectivelly. The rats were weighed at the intervals of five days for a period of fortytwo days in order to see the effect of chloroquine administration on growth. This was possible by comparing the weights of a group of rats in a control experiment. The group was kept in the metabolic cages in the same manner as the experimental animals without chloroquine administration.

Identification of chloroquine and its metabolites in the urine. A 0.1% solution of chloroquine and each of its metabolites were prepared in 0.1 M hydrochloric acid. 10ml of each of these solutions was placed in a 50ml separating funnel, 0.5ml of 10% sodium hydroxide added, mixed well and them 19ml of chloroform was added. The mixture was shaken for five minutes and the chloroform layer was removed carefully using a pipetor. The extraction was repeated with another 10ml of chloroform. The chloroform extracts were pooled together, evaporated and 2ml methanol added. This

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procedure was repeated for all the metabolites and these solutions served as the standard for the identification of the metabolites in the samples.

The samples SO, S1, S3, S5, S7, S28 and S42 (where SO, S1, S3, S5, S7, S28 and S42 are urine samples collected at zero day and after 1,3,5,6,28 and 42 days respectively) were extracted with coloroform and treated as the standard solutions. The sample solutions were spotted along with the standard solution on 0.5mm thickness silica Gel G (type 60) plate using capillary tube. The spots were allowed to dry and developed in 98.5: 1.5 methanol/ammonia mixture in TLC tanks with lids. After one hour of development, the plates were allowed to dry in air, then in an oven and the spots were identified using ultraviolet light at 254nm. From the emerging spots the R_f values for the standards were calculated which were used to identify the metabolites present in the urine of these rats.

Quantitative determination of chloroquine and its metabolites in the urine. Chloroquine and its metabolites were determined by scrapping each of the circled spots and dissolving in 10ml of ethanol; the solution was centrifuged and 5ml of the supernatant layer taken for analysis using the spectrofluorimetric technique (Adelusi and Salako 1980). Each spot was analysed for chloroquine, desethylchloroquine bisdesethylchloroquine and the 4-amino-7-chloroquinoline respectively.

Results and Discussion

Table 1 shows the R_f values of chloroquine and its major metabolites. The values obtained are the means of 5 determinations with standard error of the means. This table enabled us to identify the metabolites of chloroquine in different samples collected at different times. Since we have determined the R_f values of the spots which appeared on the thin layer chromatographic plate, it is therefore unnecessary to present the picture of the plate. The metabolites obtained in this study were identified from the results (Tables 1-2). It can be seen (Table 2) that only one metabolite, that is the desethylchloroquine, was identified after one day of chloroquine administration. An additional metabolite, 4-amino-7-chloroquine was identified after the third and the fifth days respectively. After the seventh day, both chloroquine and the desethylchoroquine were identified. After 28 days, only chloroquine was identified (Table 2), in a small amount of 0.23 ± 0.01 ug ml^{-1}. Neither chloroquine nor its metabolites were identified six weeks after the experiment.

Table 2 shows the concentrations of chloroquine and metabolites and the values presented are the means of five determinations with the standard error of the means. It can be seen (Table 2) that considerable amount of chloroquine was excreted after the first and the third days. At the fifth day,

Table 1
The R_f values for chloroquine and its metabolites in the urine of the rats.

R_f values of chloroquine and its metabolites	
Drug	R_f Values
Chloroquine (CQ)	0.40 ± 0.01
Desethylchloroquine (DG)	0.49 ± 0.02
Bisdesethylchloroquine (BQ)	0.55 ± 0.02
4-amino-7-chloroquine (4-A-7 chloroquinoline)	0.62 ± 0.03
SO (Urine before C Q administration)	No spot was identified
S1 Urine samples after 1 day	0.39 ± 0.01,0.49 ± 0.01
S3 Urine samples after 3 days	0.41 ± 0.02, 0.49 ± 0.01;0.61± 0.02
S5 Urine samples after 5 days	0.40 ± 0.01,0.50 ± 0.02, 0.62 ± 0.02
S7 Urine samples after 7 days	0.39 ± 0.02, 0.50 ± 0.01
S28 Urine samples after 28 days	0.40 ± 0.01
S42 Urine amples after 42 days	No spot was identified

Table 2
Chloroquine and its metabolites in the urine of rats.

Days	Chloroquine (ug ml^{-1})	Desethyl-chloroquine (ug ml^{-1})	4 - amino-7-chloroquinoline (ug ml^{-1})
0	--------	---------	---------
1	235.00 ± 12.00	100.00 ± 8.20	---------
3	150.01 ± 12.00	43.00 ± 5.00	15.00 ± 2.10
5	45.23 ± 10.00	24.22 ± 2.50	8.33 ± 1.00
7	7.00 ± 0.11	8.15 ± 0.90	---------
28	0.23 ± 0.01	---------	---------
42	--------	--------	---------

the value decreased to less than one third of the value obtained on the third days. The amount of 4-amino-7-chloroquinoline excreted was quite low compared with the amount of chloroquine and the desethylchloroquine.

It follows from the results (Table 2) that the major metabolites of chloroquine is the desthylchloroquine and this is in agreement with previous report (Kuroda 1962). The 4-amino-7-chloroquinoline identified on the third day and the fifth day was not found on the seventh day, and is therefore a minor metabolite (Kuroda 1962).

The implication of these results is that only two metabolites of chloroquine are present in the urine of the rats, desethylchloroquine and 4-amino-7-chloroquinoline.

The desethylchloroquine was in a higherconcentrations. which was present in the urine even after four weeks at a concentration of 0.23 µg $24h^{-1}$. No 4-amino-7 chloroquinoline was found after a week. The bisdesethylchloroquine, one

of the metabolites identified by previous investigators (Essien 1978) in traces in the urine of human subjects was not found in this study. Probably due to the fact that the amount detected might have not been formed at all. The presence of chloroquine after four weeks was in conformity with previous report that chlorquine was highly tissue found (Adelusi and Salako 1982).

The detection of same type of metabolities in the urine of these rats as that in the human urine probably indicates that man and animal metabolise chloroquine in a similar way. However, the amount of metabolites may be smaller in these rats than in the man.

Since it is unusual to extrapolate animal studies to man, it will be necessary to carry out the studies to determine the amount of chloroquine and its metabolites in man.

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