Pakistan J. Sci. Ind. Res., Vol. 30, No. 9, September 1987

BLOOD AND TISSUE LEVELS OF CHLOROQUINE IN PROTEIN ENERGY MALNOURISHED RATS AFTER PROPHYLAXIC TREATMENT

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(Received January 9, 1985; revised July 22, 1987)

The tissue and blood levels of chloroquine were studied in the normal and protein energy malnourished (P.E.M) rats for a period of twelve weeks after the administration of a weekly dose of 10 mg/kg given intraperitoneally. It was found that the high perfused tissues (liver, kidney, spleen, lungs and heart) attained saturation in the P.E.M. rats whereas the level of chloroquine in the low perfused tissues (skeletal muscle, eye and skin) and the blood increased progressively.

In the normal rats, none of the tissues got to saturation; the tissue and blood levels increased with time. The brain did not show any defined pattern in both normal and the P.E.M. rats. It was therefore suggested that prolonged administration of chloroquine would lead to accummulation of the drug in these diseased states.

Key words: Blood and tissue level; Chloroquine; Malnourished rats; Prophylaxic treatment.

INTRODUCTION

Adelusi and Salako [1] have studied the blood and tissue levels of chloroquine in the normal rats after prolonged administration of the drug. They found that there was no saturation in any of the tissues and the blood levels after given chloroquine at a dose of 10 mg/kg weekly for 28 weeks.

Studies on the absorption, distribution and elimination of chloroquine in relation to dietary protein [2] showed that the absorption, distribution and elimination of chloroquine in protein energy malnourished (rats) were impaired. Another study on the urinary excretion of chloroquine in relation to dietary protein also revealed that the metabolism of chloroquine was impaired in the PEM rats [3].

In view of the above findings and of the fact that PEM, being a disease of the tropics where chloroquine is indiscriminately administered through self medication either as prophylactic in radical curatives, it is necessary to study the tissue and the blood levels of chloroquine in the PEM diseased state after long-term administration using the rat as a model.

MATERIALS AND METHODS

The investigation was conducted on weanling male albino rats of the wister strain aged between 3-4 weeks and weighing between 26-34 g. The rats were divided into two groups, the first group was used as the normal rats and protein energy malnutrition (PEM) was induced in the second group. The method for inducing PEM in these rats has been previously described [2]. This involved feeding the rats with a diet which has 2 % protein, 82 % carbohydrate, 8 % corn oil as fat, 4 % mineral salt and 4 % vitamin mixture. The normal rats were fed on standard diet, they were weighed every week along with the PEM rats and chloroquine at a dose of 10 mg/kg in normal saline was administered intraperitoneally every week for a period of 12 weeks. Within the normal and the PEM rats were the control rats and normal saline without chloroquine was administered to these rats.

The experimentation was carried out as previously described [1], that is, at the ends of weeks 1-6, 8 and 12 weeks, six PEM rats from each of the chloroquine treated groups and one from each of the control groups were slightly anaesthesised with ether and 2-3 ml blood sample withdrawn by cardiac puncture into a lithium heparin bottle, centrifuged at a speed of 2,500 rpm. for 5 min. The plasma and the red blood cells were separated and stored at -10° until analysed. The rats were finally killed and the liver, lungs, kidneys, spleen, heart, eyes, brain, a sample of the abdominal skin and the muscle removed, blotted dry and also stored at -10° until analysed. These procedures were repeated for the normal rats.

Determination of chloroquine in the specimens. The tissues were allowed to thaw and blotted dry. Samples of the tissues (from the whole tissue) of about 0.5 g were

weighed accurately and homogenised in 5-10 ml 0.1 M HCl with an electrically driven homogeniser; the whole eye was weighed and used for analysis. The homogenate was centrifuged and the supernatant layer used for analysis. For the red blood cells, 1.0 ml of the packed cell was accurately measured out and diluted to 5 ml with 0.1 M HCl, shaken well and centrifuged and the supernatant layer also used for analysis.

1.0 ml of the tissue homogenate, plasma and prepared red blood cells were analysed by the spectrofluorimetric method of Rubin *et al.* [4] as modified by Adelusi and Salako [5]. This method of analysis of chloroquine has a lower limit of sensitivity of 5 ng/ml and a percentage recovery of over 95. Chloroquine sulphate used in the studies was a gift from May and Baker (Dagenham, UK) and the quantiities administered are given in terms of the base.

RESULT

Fig. 1 shows the tissue and blood levels of chloroquine in the PEM rats with respect to time, while Fig. 2 shows the levels of chloroquine in the tissues and blood of the normal rats. In Fig. 1, the high perfused tissues (liver, kidney, heart, lungs and spleen) got to saturation between 6-12 weeks. The values for these tissues at 1, 6 and 12 weeks are presented in Table 1. This table shows (by statistical analysis) that the concentrations of chloroquine in these tissues increased between 1 to 6 weeks ($P < 0.01$) but between 6 to 12 weeks, there are no significant difference in the concentration of chloroquine in these tissues ($P > 0.05$).

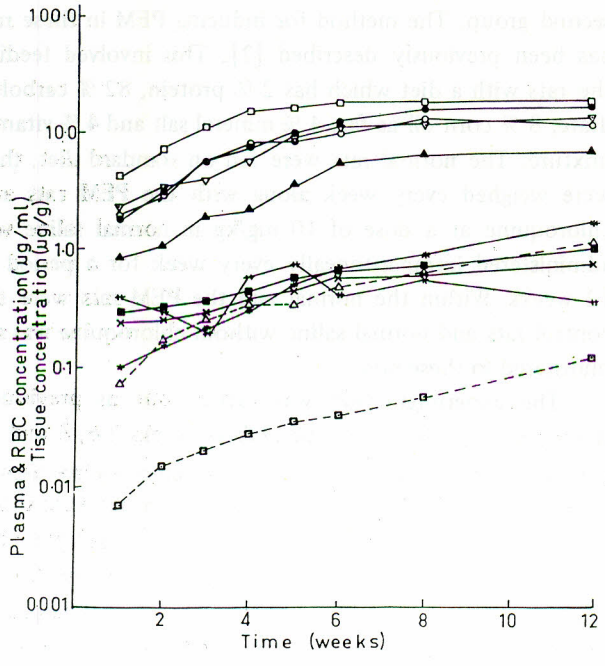

Fig. 1. Tissue and blood levels of chloroquine in the kwashiorkor rat with respect to time.

Liver	(□ – □)	Kidney	(● – ●)
Lungs	(▽ – ▽)	Spleen	(△ – △)
Heart	(▲ – ▲)	Brain	(H – H)
Eye	(■ – ■)	Skeletal Muscle	(* – *)
Skin	(X – X)	RBC	(△ – △)
Plasma	(□ – □)		

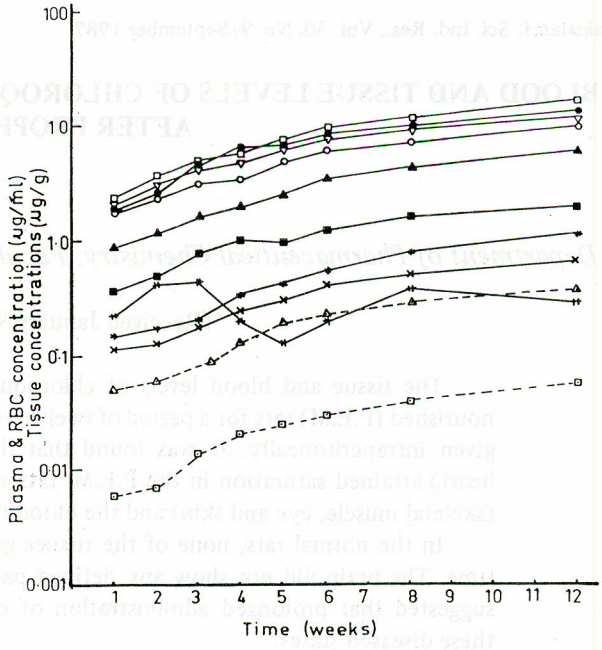

Fig. 2. Tissue and blood levels of chloroquine in the normal rat with respect to time.

Liver	(□ – □)	Kidney	(● – ●)
Lungs	(△ – △)	Spleen	(△ – △)
Heart	(▲ – ▲)	Brain	(H – H)
Eye	(■ – ■)	Skeletal Muscle	(* – *)
Skin	(X – X)	RBC	(△ – △)
Plasma	(□ – □)		

This study would have been carried on for 28 weeks as the previous study on normal rats [1] but after 13 weeks from the commencement of the experiment, some of the PEM rats died and in fact none were left after 14 weeks.

By the same statistical analysis using students' t-test, the low perfused tissues (skeletal muscle, skin and eye) did not get to saturation in the PEM rats. The chloroquine levels in these tissues increased progressively. The brain did not show any regular pattern in both the normal and the PEM rats (Fig. 1 and 2). The blood levels of the PEM rats (red blood cell and plasma levels) increased rapidly when the high perfused tissues got to saturation (Fig. 1).

In Fig. 2, none of the tissues and blood levels got to saturation. The concentration of chloroquine increased progressively in all tissues except in the brain where the

Table 1. Concentration of chloroquine (μg/g) in the high perfused tissues of protein energy malnourished (PEM) rats.

Organs	Weeks		
	1	6	12
Liver	4.5 ± 0.6	19.0 ± 2.2	20.0 ± 2.4
Kidney	1.9 ± 0.3	13.0 ± 1.6	16.0 ± 1.9
Lung	2.1 ± 0.4	10.0 ± 1.4	11.2 ± 1.3
Spleen	2.5 ± 0.3	11.1 ± 1.7	10.5 ± 1.9
Heart	0.86 ± 0.9	6.0 ± 0.8	5.7 ±

Table 2. Concentration of chloroquine (μg/g) in the high perfused tissues of normal rats.

Organs	Weeks		
	1	6	12
Liver	2.4 ± 0.3	10.0 ± 1.4	16.0 ± 1.8
Kidney	1.8 ± 0.2	8.5 ± 0.6	13.8 ± 1.3
Lung	1.9 ± 0.3	6.0 ± 0.8	10.0 ± 1.2
Spleen	2.0 ± 0.2	7.5 ± 0.6	10.8 ± 1.1
Heart	0.9 ± 0.1	3.5 ± 0.4	6.0 ± 0.5

concentration of chloroquine did not show any regular pattern with time. Table 2 shows the values for the high perfused tissues at 1, 6 and 12 weeks and from this table it can be seen that the concentration of chloroquine increased progressively from 1 to 6 weeks and so on to 12 weeks ($P < 0.01$).

The comparison of the tissue and blood levels of chloroquine in both the normal and the PEM rats using the students t-test showed that for the low perfused tissues, except for the first week, the chloroquine level in the eye of the normal rats was higher than those of the PEM rats ($P < 0.05$). The other tissues such as the brain, skeletal muscle and the skin showed higher values in the PEM rats. The plasma and the red blood cell chloroquine levels were also higher in the PEM rats than in the normal rats ($P < 0.05$). The high perfused tissues showed higher chloroquine levels in the PEM rats than in the normal rats ($P < 0.05$).

DISCUSSION

Various workers [6-11] have studied the chronic administration of chloroquine in the rats, the dose given ranged from 25 mg/kg to 40 mg/kg for a varying period of 10 days to 24 weeks. The frequency of dosing varied from once a week to six times in a week for these periods of time. The time when the tissues got to saturation also varied from one experiment to another. In the previous study by Adelusi and Salako [1], it was found that the normal prophylactic dose of chloroquine given for 28 weeks in rats did not lead to saturation in any of the tissues studied and the blood levels were still rising. In the present investigation, chloroquine at the prophylaxic dose of 10 mg/kg similar to those normally given to man was administered to PEM rats in order to see if any of the tissues would get to saturation following the previous report on the impairment of the metabolism of chloroquine in the PEM state [2,3,12]. In the normal rats, the results obtained was similar to those obtained in the previous study [1] but the tissues and the blood levels of chloroquine in the PEM rats were quite different from those obtained for the normal rats since some of the tissues such as liver, kidney, heart, spleen and lung got to saturation in the PEM rats whereas tissues such as the skeletal muscle, eye and the skin did not get to saturation in these rats.

The rate of increase of chloroquine concentration in the tissues that were not saturated in the case of the PEM rats were more rapid than when they have not reached saturation. The fact that the PEM rats did not survive for more than 13 weeks could be attributed to the poor condition of these rats caused by the effect of the disease and moreover the saturation obtained in the heart could play some role since a previous report [13] has shown that chloroquine is cardiotoxic; therefore it could be assumed that the death of these rats might be due to the cardiotoxicity caused by chloroquine, although this might not be the only effect since other factors such as liver failure [14], anaemia and severe hypoglycaemia [15,16] have been shown to cause death in PEM state.

Previous workers [11] who administered chloroquine in larger doses to albino rats found that tissues such as the spleen, liver, heart and lung got to saturation whereas chloroquine in the other tissues such as the skeletal muscle increased progressively. In the present study, the tissues of the normal rats did not get to saturation probably suggesting that more chloroquine was needed for saturation than was given in this study. In the case of the PEM rats, some tissues such as liver, kidney, heart, spleen and the lung got to saturation, but according to Buchanan and Van der Walt [17] who found that more chloroquine was bound in serum from PEM state than in the normal serum, resulting in a lower fraction of free chloroquine, one would expect lower chloroquine tissue concentrations since only the free drug is capable of entering the tissues. However,

severe pathophysiological changes and reduced metabolism might be responsible for higher chloroquine concentration in the PEM rats, thereby leading to saturation in some of the tissues studied.

The results from this investigation suggest that in PEM state, prophylaxic administraion of chloroquine for a prolonged period would lead to high blood level of the drug. Although it is sometimes difficult to extrapolate from animal studies to man, yet the results from this investigation suggest the need for such a study in man.

Acknowledgement. I greatly acknowledge the support of the Drug Research Unit of the Biode Building, UCH, Ibadan for allowing me to use its spectrofluorimeter for the analysis of chloroquine in the samples.

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