Br. J. Pharmac. (1971), **43**, 335–340.

Chloroquine concentrations in the skin of rabbits and man

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Summary

1. Chloroquine was given by a single intravenous injection to rabbits and to two groups of patients. The concentrations of the drug and its more polar metabolites in plasma and tissue were measured by a fluorometric method.

2. The concentrations of chloroquine in rabbit liver exceeded those in skin and these in turn exceeded those in plasma. Chloroquine concentrations in rabbit skin were fairly steady between 24 and 72 h after injection. Chloroquine was still present in skin 30 days after the injection. Metabolites of chloroquine were present in plasma, skin and liver.

3. Skin was taken from patients 48 h after an intravenous dose of chloroquine (1·25 mg/kg). Skin from patients prone to chloroquine-induced pruritus contained higher concentrations of chloroquine ($P<0·01$) and lower concentrations of metabolites ($P<0·01$) than skin from other patients.

4. It is suggested that increased liability to chloroquine-induced pruritus may be associated with a lower rate of chloroquine metabolism.

Introduction

The 4 aminoquinoline derivative, chloroquine, is widely used in the treatment of malaria and in a variety of unrelated diseases (Rees & Maibach, 1963; Knox & Owens, 1966). Certain patients develop a characteristic pruritus during chloroquine administration (Ekpechi & Okoro, 1964; Olatunde, 1969; Olatunde, 1970).

Chloroquine is concentrated in many tissues including liver, lung and kidney (Berliner, Earle, Taggart, Zubrod, Welch, Conan, Banman, Scudder & Shanon, 1948), the retina and choroid of pigmented rabbits (Rubin, Zvaifler, Bernstein & Mansour, 1965) and the skin (McChesney, Nachod & Tainter, 1957; Schaffer, Cahn & Levy, 1958). The chloroquine concentration in skin may be greater when the melanin content is greater (Zvaifler, Rubin & Bernstein, 1963; Sams & Epstein, 1965). However, Tuffanelli, Abraham & Dubois (1963) found no differences between the concentrations of chloroquine in the skin of negro and white patients.

The purpose of this study was to determine whether patients who had previously developed pruritus during chloroquine therapy showed an abnormal capacity to concentrate chloroquine in the skin.

Methods

Animal experiments

Chloroquine sulphate (May & Baker Ltd., 5 mg base/kg body weight) was injected into the ear veins of Dutch pigmented (DP) and New Zealand Albino (NZA) rabbits weighing about 2 kg and aged about 10 weeks. The rabbits were killed at intervals after injection which varied from 1 h to 30 days. Samples of blood, liver and skin were taken for the measurement of the concentrations of chloroquine and its more polar metabolites. Control samples were taken from untreated animals.

Blood obtained by slitting the throat was collected in bottles containing potassium oxalate and centrifuged immediately at 1,500 r.p.m. for 30 min; 2 ml of the plasma was taken for assay of chloroquine and metabolites.

Samples of liver of about 1 g were weighed and homogenized in 5–10 ml 0·1 N HCl with an electrically driven homogenizer. The homogenate was centrifuged at 3,000 r.p.m. and 2 ml of the clear supernatant was taken for assay.

Shaved skin was removed from the right half of the dorsum of the trunk. Subcutaneous fat was removed and about 1 g of skin was weighed and homogenized as described for liver.

Human studies

Twelve Nigerian patients (West African negroes) who had received chloroquine for the treatment of malaria were studied. Six of the patients had developed the characteristic pruritus during chloroquine therapy. The other six had not developed pruritus; these included six males and six females, with equal sex distribution in each group. The studies were explained in detail and the patients gave informed consent. The ages ranged from 14 to 51 years with a mean of 32·7 years. A patient was assigned to the pruritus group if (a) he developed itching with no visible skin lesion (other than those due to scratching and rubbing) within 24 h of chloroquine therapy without a current history of itching, dermatitis or predisposing factors to pruritus, and showed no pruritus reaction to a placebo; (b) he had a history of mild to moderate itching which became more severe after chloroquine administration, provided he had no predisposing factors to itching such as liver, disease, neoplasm, diabetes mellitus, filariasis helminthic infection or any form of skin lesion. The patients studied were not hospitalized but each was seen 5 times within 3 weeks. On the first visit detailed information was given to any patient selected for the study; he was instructed not to take antimalarial chemotherapy or antipyretic analgesic throughout the period of investigation. A patient who had thus given informed consent reported a week later for the control samples.

Control samples of venous blood and about 50 mg of lateral mid thigh skin were taken for the estimation of the concentration of residual chloroquine and metabolites. Five days later a single dose of chloroquine sulphate (1·25 mg base/kg of body weight) was injected into an antecubital vein. Blood and skin samples were taken again 48 h after the injection. The samples were prepared for the assay of chloroquine and metabolites in the same way as the samples of rabbit tissue. A 48 h interval between the intravenous dose and the repeat skin biopsy was chosen because

the chloroquine concentrations in rabbit skin appeared to be fairly steady between 24 and 72 h (Table 1). The last visit for each patient was 1 week after the test study to ensure adequate healing of the skin biopsy sites.

Measurement of chloroquine and metabolites

Chloroquine and its more polar metabolites were measured in extracts of tissues by spectrophotofluorometry (Brodie, Udenfriend, Dil & Chenkin, 1947; Rubin, Bernstein & Zvaifler, 1963). Replicate measurements were made on each sample.

Standard solutions of pure chloroquine sulphate were prepared in 0·1 N HCl. Two ml aliquots of HCl, chloroquine standard, plasma or tissue homogenate were added to 2·0 ml 0·1 N NaOH in 50% ethanol (v/v) in a graduated centrifuge tube. The mixture was made up to 6·0 ml with absolute ethanol, shaken vigorously for 1 min and centrifuged at 2,500 r.p.m. for 5 minutes. Three millilitres of the supernatant were added to 20 ml dichloromethane and shaken for 20 min in a 50 ml

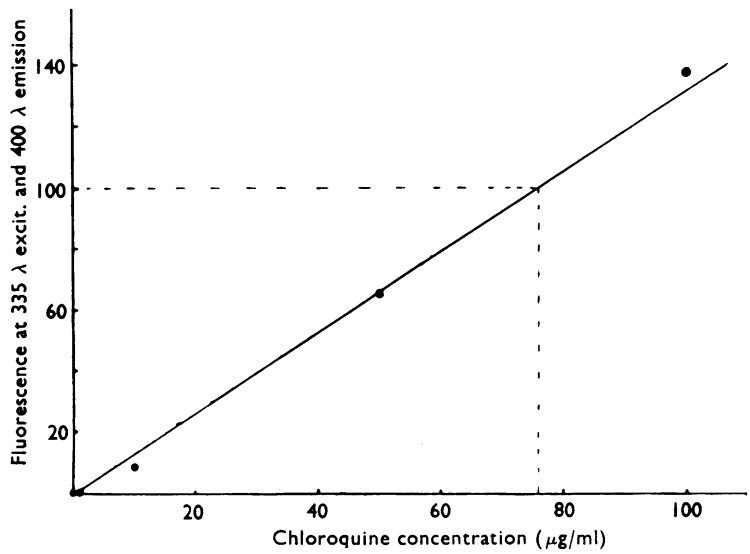

FIG. 1. Fluorescence at 335λ excitation and 400λ emission to standard chloroquine concentrations, using the Hitachi Spectrophotofluorometer. See also Table 1. From the graph, a fluorescence reading of 100 is equivalent to a chloroquine concentration of 76 μg/ml.

TABLE 1. *Distribution of chloroquine in plasma, skin and liver of pigmented (DP) and albino (NZA) rabbits given a single intravenous dose of chloroquine (5 mg/kg body weight)*

Time interval after intravenous chloroquine before animals were killed	Concentrations of chloroquine: Plasma (μg/ml)		Skin (μg/g)		Liver (μg/g)	
	DP	NZA	DP	NZA	DP	NZA
Control	0·4	0·4	0·1	0·7	0·3	0
1 h	3·0	3·5	36·8	24·0	148	121
6 h	0·1	0·1	2·6	3·8	40·3	37·2
24 h	0·8	0·2	12·1	18·4	189	226
48 h	0·4	0·2	10·3	10·4	216	157
72 h	0·3	1·2	9·4	9·0	157	185
4 Days	0·1	0	0	0	238	156
7 Days	4·8	1·4	0	0	36·3	24·6
14 Days	0·7	0·4	2·8	4·4	29·3	8·2
30 Days	0·7	0·6	2·4	16·8	0	1·0

stoppered centrifuge tube. The aqueous layer was removed and discarded. The organic layer was washed with 15 ml borate buffer, pH 9·5.

Chloroquine assay

Fifteen millilitres of the organic layer was shaken with 10 ml dipotassium borate buffer pH 7·85 for 10 minutes.

Ten millilitres of the organic layer were shaken with 5 ml of 0·1 N HCl for 15 min; 3 ml of the acid layer were mixed with 3 ml 0·2 N NaOH in 50% ethanol (v/v). This solution was placed in the fluorimeter and the intensity of the emission at 400 nm was measured using an excitation wavelength of 335 nm.

Chloroquine metabolites assay

Three millilitres of the dipotassium borate buffer layer were mixed with 3 ml 0·1 N NaOH in 50% ethanol (v/v), and the solution was read in the fluorimeter as above.

A calibration curve was determined with standard solutions of chloroquine. The chloroquine metabolites were not individually characterized but were estimated in terms of unchanged chloroquine base. Each assay sequence included a blank and four standards (Fig. 1).

Results

Animal experiments

The highest plasma concentrations of chloroquine were observed in the 1 h sample (Table 1). The skin concentrations at this time were greater by a factor of about 10 and the liver concentrations by a factor of about 50. During the first 72 h the con-

TABLE 2. *Distribution of chloroquine metabolites in plasma, skin and liver of DP and NZA rabbits given a single intravenous dose of chloroquine (5 mg/kg body weight)*

Time interval after intravenous chloroquine before animals were killed	Concentrations of chloroquine metabolites: Plasma (μg/ml)		Skin (μg/g)		Liver (μg/g)	
	DP	NZA	DP	NZA	DP	NZA
Control	0·2	0·9	1·5	0	0	0
1 h	0·4	0	0	1·7	5·9	9·5
6 h	0·0	0·0	0·1	0·1	3·6	3·8
24 h	0	0	0	0	23·3	26·0
48 h	0·6	0·2	0·1	0	12·4	11·1
72 h	1·5	1·6	20·0	10·5	28·6	26·3
4 Days	0	0	3·1	5·1	32·0	17·8
7 Days	0·9	2·5	0·9	0	8·3	0
14 Days	1·1	0·7	0·7	5·5	3·1	6·7
30 Days	2·2	1·7	4·1	0·4	1·8	0

TABLE 3. *Concentrations of chloroquine in plasma (μg/ml) and skin (μg/g) before and 48 h after an intravenous dose (1·25 mg base/kg), (a) in patients prone to chloroquine pruritus and (b) in patients not prone to pruritus*

	Before intravenous chloroquine		
	(a) Conc. ±S.E.M. *n*	(b) Conc. ±S.E.M. *n*	*t* test *P*
Plasma	1·4± 0·6 6	1·5± 0·4 6	>0·99
Skin	83·8±10·6 6	41·5±16·8 6	>0·05
	48 h after intravenous chloroquine		
	(a) Conc. ±S.E.M. *n*	(b) Conc. ±S.E.M. *n*	*t* test *P*
Plasma	3·3± 1·7 6	2·3± 0·4 6	>0·50
Skin	112·0±13·0 6	40·8±11·8 6	>0·01

The significance of the difference between the values for *a* and *b* is indicated by the value of *P* in Student's *t* test.

centrations in plasma and skin declined but the concentrations in liver continued to rise until the latter was greater than the plasma concentration by a factor of 200 or more. Chloroquine was still detected in the plasma and tissues 30 days after the single intravenous dose.

The concentrations of chloroquine in the skin of the pigmented rabbits were of the same order as in the albino rabbits.

The estimates of metabolite concentration (Table 2) were very variable but tended to be the highest in all tissues between days one and four.

Human studies

The concentration of chloroquine in the skin of the two groups of patients are shown in Table 3. There was no significant difference in the apparent concentrations of chloroquine before the patients had received the intravenous dose. Forty-eight hours after the intravenous dose, however, the concentration of chloroquine in the skin was higher in the group which was prone to chloroquine pruritus ($P<0{\cdot}01$, two tailed t test; $P<0{\cdot}01$, Mann Whitney U test).

The concentrations of chloroquine in plasma were comparable in the two groups of patients before and after intravenous chloroquine (Table 3).

The concentrations of chloroquine metabolites in the skin of the two groups of patients are shown in Table 4. There was no significant difference in the apparent concentrations of chloroquine metabolites before the patients had received the intravenous dose. Forty-eight hours after the intravenous dose, however, the concentration of chloroquine metabolites in the skin was lower in the group which was prone to chloroquine pruritus ($P<0{\cdot}01$, two tailed t test; $P<0{\cdot}01$, Mann-Whitney U test).

The concentrations of chloroquine metabolites in the plasma were comparable in the two groups of patients before and after intravenous chloroquine (Table 4).

Discussion

Patients prone to develop pruritus during treatment with chloroquine have fairly high concentrations of unchanged chloroquine and fairly low concentrations of polar metabolites in the skin 48 h after an intravenous dose.

Samples of human skin taken before the intravenous dose contained chloroquine

TABLE 4. *Concentrations of chloroquine metabolites in plasma (μg of chloroquine equivalent/ml) and skin (μg of chloroquine equivalent/g) before and 48 h after an intravenous dose (1·25 mg base/kg), (a) in patients prone to chloroquine pruritus and (b) in patients not prone to pruritus*

Before intravenous chloroquine

	(a) Conc. ± S.E.M.	*n*	(b) Conc. ± S.E.M.	*n*	*t* test *P*
Plasma	2·0 ± 1·0	6	1·1 ± 0·6	6	>0·40
Skin	25·2 ± 7·4	6	45·8 ± 14·5	6	>0·20

After intravenous chloroquine

	(a) Conc. ± S.E.M.	*n*	(b) Conc. ± S.E.M.	*n*	*t* test *P*
Plasma	3·7 ± 1·5	6	5·4 ± 1·0	6	>0·30
Skin	30·3 ± 6·1	6	89·7 ± 14·4	6	>0·01

The significance of the difference between the values for *a* and *b* is indicated by the value of *P* in Student's *t* test.

and metabolites or compounds with similar fluorescence characteristics. These were attributed to the administration of preparations containing chloroquine not less than 4 weeks previously. Samples of skin from untreated rabbits did not give high fluorescence readings.

It is tentatively suggested that patients prone to develop chloroquine pruritus may have a slower rate of chloroquine metabolism. A similar suggestion was made by Shee & Barnard (1963) to account for increased liability to skin pigmentation in patients receiving amodiaquine, chloroquine, hydroxychloroquine or mepacrine.

Professor Dinah James of the Department of Pharmacology, University of Ibadan, Professor H. Schneiden and Dr. G. Mawer of the Department of Pharmacology, University of Manchester, Dr. G. O. Alabi, Dermatologist, University College Hospital, Ibadan and Dr. G. O. Ojo, Physiology Department, University of Ibadan, cooperated and gave advice. Part of the work was done while I was a Commonwealth Scholar in the Department of Pharmacology, University of Manchester. The assistance of the West African Council for Medical Research in the purchase of certain equipment is gratefully acknowledged. This work forms part of my M.D. thesis, University of London.

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(*Received December* 2, 1970)