J. Pharm. Pharmacol. 1982, 34: 733–735
Communicated February 22, 1982

0022–3573/82/110733–03 $02.50/0

Tissue and blood concentrations of chloroquine following chronic administration in the rat

S. A. Adelusi *L. A. Salako, *Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria*

The antimalarial drug, chloroquine, is extensively distributed in tissue and slowly eliminated such that after a single dose, a plasma half-life of 3–5 days has been found (McChesney & McAuliff 1961; McChesney et al 1967). A peak tissue/plasma concentration ratio greater than 300 is obtained in many tissues and after a single dose the drug can be found in the liver and urine for up to five years (Gaudette & Coatney 1961; Rubin et al 1963; Zvaifler et al 1963).

Chronic administration of chloroquine for the treatment of rheumatoid arthritis has revealed an ocular toxicity due to accumulation of the drug in the pigmented layers of the eye, particularly the choroid (Fuld & Horwish 1958; Fuld 1959). A more recent indication for chronic administration of chloroquine is in the prophylaxis of malaria, for which the drug is administered at a dose of 300–600 mg weekly to adults. The long term toxic effects of chloroquine when administered in this way are unknown but no ocular toxicity has been reported even after five years of such use. Since tissue toxicity and other untoward effects are largely determined by tissue stores (Fuld & Horwish 1958; Fuld 1959) and blood levels (Laaksonen et al 1974; Frisk-Holmberg et al 1979) of the drug, it is useful to know the changes occurring in tissue and plasma concentrations during chronic administration. Previous studies in animals have given conflicting results. McChesney et al (1965) found a steady increase in the tissue and plasma concentrations in rats throughout a 3-month period although the increase was fastest in the first month. Grundmann et al (1972) found that most rat tissues were saturated with chloroquine between the 10th and the 16th weeks. Plasma concentrations were not measured hence the effect of tissue saturation on blood levels was not determined, yet saturation of tissue stores would be expected to lead to a rapid increase in plasma concentration that could affect the pattern and incidence of adverse reactions to the drug. We have reinvestigated the uptake of chloroquine by rat tissues during chronic administration of the drug and in particular to relate the tissue levels to plasma concentrations.

Materials and methods

Albino rats of the Wistar strain, 130 and 170 g, fed on standard diet were weighed and chloroquine 10 mg kg^{-1} in 0·9% NaCl (saline) or saline alone was administered intraperitoneally every week for 28 weeks.

* Correspondence.

At the end of weeks 1–6, 8, 12, 16, 20, 24 and 28, six rats from the chloroquine treated group and one from the control group were slightly anaesthetized with ether and 3–4 ml blood withdrawn by cardiac puncture into a lithium-heparin bottle, centrifuged (2500 rev min^{-1} 10 min) and the separated plasma and red blood cells stored at −10 °C until analysed. The rats were then killed and the liver, kidneys, spleen, lungs, heart, eyes, brain, a sample of abdominal skin and the gastrocnemius muscle removed, blotted dry, weighed, and also stored at −10 °C until analysed.

Drug analysis. Samples of tissues (0·5 g) were weighed accurately, homogenized in 5–10 ml 0·1 M HCl, the homogenate centrifuged and the supernatant used for analysis. 1·0 ml of the packed red blood cells was accurately measured and diluted to 5 ml with 0·1 M HCl, shaken well and centrifuged and the supernatant used for analysis.

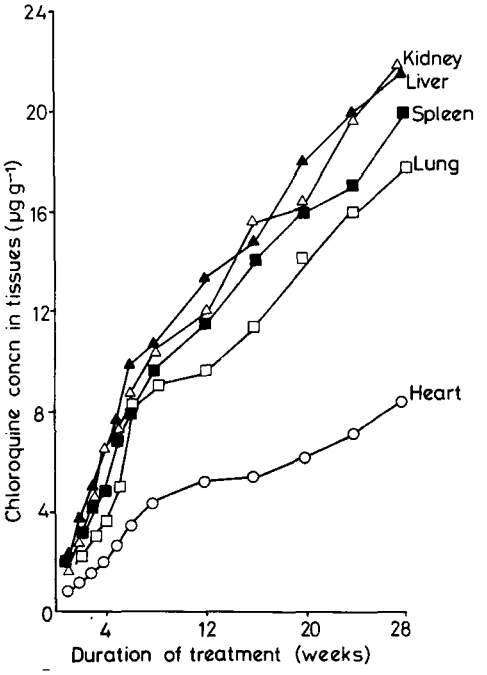

FIG. 1. Chloroquine concentration in high perfusion tissues (kidney (△) liver (▲) spleen (■), lung (□) and heart (○)) during treatment of rats with 10 mg kg^{-1} chloroquine intraperitoneally once a week. Each point represents the mean for 6 rats.

Table 1. Tissue/plasma chloroquine concentration ratios at different intervals in the high perfusion tissues. Each value was the mean for 6 rats treated with 10 mg kg^{-1} chloroquine weekly.

Week	Liver	Kidney	Spleen	Lungs	Heart
1	385·0	296·7	335·0	306·7	148·3
2	541·4	418·6	435·7	322·9	177·1
3	358·6	310·7	399·3	213·6	114·3
4	268·1	312·9	228·6	173·8	96·7
5	282·6	269·6	256·3	199·6	99·3
6	318·4	280·0	254·2	264·8	117·1
8	260·5	259·0	237·3	222·2	105·1
12	233·7	208·8	200·9	166·1	90·4
16	214·1	226·8	203·3	164·9	76·2
20	222·7	199·6	198·0	172·4	74·7
24	213·8	211·9	184·3	170·2	76·0
28	208·5	211·9	193·2	172·6	81·9

The tissue homogenates, prepared red blood cells and plasma were analysed for chloroquine by the fluorimetric method of Rubin et al (1965) as modified by Adelusi & Salako (1980). 1 ml of each sample was made alkaline with 0·2 ml ammonia solution (s.g. 0·91), extracted with diethyl ether, the extract washed with borate buffer, pH 9·5, and then shaken with borate buffer, pH 7·85, to remove metabolites. Chloroquine was then repartitioned into aqueous solution by shaking the washed ether extract with 0·1 M HCl. The acid extract was mixed with alcoholic NaOH and the fluorescence of the final solution (pH 9·9) was read on a Perkin-Elmer spectrofluorimeter using excitation and emission wavelengths of 331 and 386 nm respectively. This method has a lower limit of sensitivity of 5 ng ml^{-1} and gives a recovery rate of over 95% (Adelusi & Salako 1980). Samples from control rats were used as blanks. Chloroquine sulphate (a gift from May and Baker, Dagenham, U.K.) quantities administered are given in terms of the base.

Results

The results are summarized in Figs 1 and 2, and Tables 1 and 2. The concentration of chloroquine rose steadily in all tissues studied up to the 28th week except for brain where irregular fluctuations occurred from week to week. The highest concentrations were found in liver, kidney, spleen and lungs (Fig. 1), and the lowest concentration in plasma (Fig. 2). Erythrocytes in the first few weeks had the next lowest concentrations but in the final weeks they rose above those of brain and skin. For all the high perfusion tissues (liver, kidney, spleen and lungs), the tissue/plasma concentration ratio rose to a maximum in the second week and then declined steadily. At the 28th week, the ratio for these tissues was still about 200 (Table 1). The red blood cell/plasma concentration ratio remained at between 5·5 and 9·0 throughout (Table 2). In the low perfusion tissues (skin, skeletal muscle, brain), the tissue/plasma concentration ratios were much lower, the lowest being in skin, for up to 3 weeks and then in brain (Table 2).

Discussion

We have used doses of chloroquine similar to those recommended for prophylaxis in man (5–10 mg kg^{-1} weekly) to investigate the tissue and blood concentrations in rats of the drug during a 28-week period. Our results show that the tissues remained unsaturated with the drug and are thus similar to those of McChesney et al (1965) who gave a much higher dose (40 mg kg^{-1} per day) for 3 months and found a progressive increase in the tissue and plasma concentrations throughout the period. Our results differ from those of Grundmann et al (1972) who gave 30 mg kg^{-1} chloroquine daily to rats over 24 weeks and found that, with the exception of skeletal muscle and brain, most tissues were saturated with chloroquine between the 10th and the 16th weeks. Both groups of authors used clearly toxic doses and it is possible that if McChesney et al had continued administration for longer than 3 months (as did Grundmann et al) tissue saturation could have been observed. Our failure to observe tissue saturation after 28 weeks of treatment suggests that the dose was not large enough to produce tissue saturation within the period.

The results of McChesney et al (1965) showed chloroquine tissue concentration to fall by over 70% in 9 days and by over 90% in 15 days on stopping the drug. This rapid fall in tissue concentration might contribute to the failure to observe tissue saturation in our study in which the drug was administered weekly and tissue specimens were taken 7 days after a dose.

Brohult et al (1979) determined plasma chloroquine

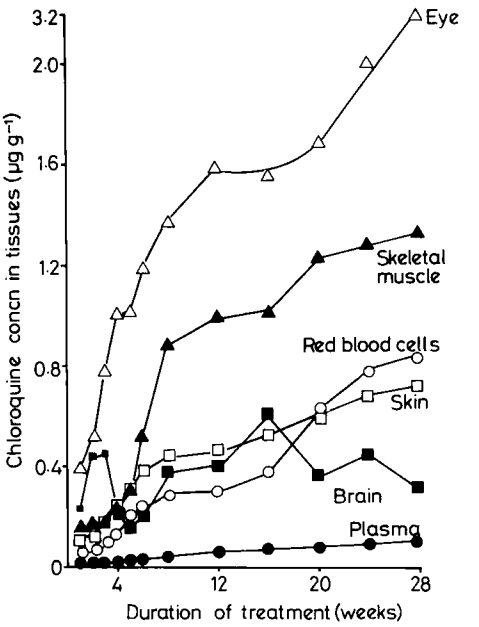

FIG. 2. Chloroquine concentration in plasma (●) red blood cells (○) and low perfusion tissues (Brain (■), Skin (□), Skeletal muscle (▲) and eye (△)) during treatment of rats with 10 mg kg^{-1} chloroquine intraperitoneally once a week. Each point represents the mean for 6 rats.

Table 2. Tissue/plasma chloroquine concentration ratios at different intervals in low perfusion tissues and red blood cells (RBC). Each value was the mean for 6 rats treated with 10 mg kg^{-1} chloroquine weekly.

Weeks	Eye	S.M.*	Skin	Brain	RBC
1	63·3	25·0	18·3	38·3	8·3
2	74·3	24·3	18·6	62·9	9·0
3	55·7	12·9	12·9	32·1	6·5
4	48·1	11·4	11·9	10·5	6·4
5	37·4	11·5	11·9	5·2	7·6
6	38·1	16·8	12·3	6·8	7·5
8	33·4	21·5	10·7	9·3	7·0
12	27·7	17·2	8·1	7·0	5·5
16	22·6	14·6	7·7	8·8	5·6
20	20·9	15·2	7·7	4·4	7·8
24	21·5	13·8	7·3	4·8	8·4
28	21·3	12·9	7·0	3·1	8·2

* Skeletal muscle.

concentrations during prophylactic administration of the drug in man. At 300 mg chloroquine per week (approximately 5 mg kg^{-1} body weight), a steady state plasma concentration was reached in 6 weeks. The plasma concentration 24 h after each dose remained below 250–400 ng ml^{-1} above which level a higher incidence of side effects has been reported (Laaksonen et al 1974; Frisk-Holmberg et al 1979). When the dose of chloroquine was doubled, the plasma concentration 24 h after each dose was above the reported threshold for increased side effects. We have failed to confirm in rats the steady state plasma concentration observed in man after 6 weeks of weekly administration of 5 mg kg^{-1} chloroquine. It is presumed that tissue store saturation did not occur in the human studies since saturation of tissue stores would most likely lead to a big rise in plasma concentration.

The concentration of chloroquine in the eye, in our study, is similar to that in other low perfusion tissues and much less than in the liver, kidney, spleen and heart. The concentration applies to the entire eye and the concentration in different fractions may differ (Lindquist 1973). However, it is well known that the highest concentrations of chloroquine are found in melanin-containing tissues. The relatively low values in the eye and skin in our study may be due to the fact that we used albino rats in which the concentrations of melanin are low (McChesney et al 1965).

This investigation received support from the UNDP/ World Band/WHO Special Programme for Research and Training in Tropical Diseases. We are grateful for technical assistance by Mrs R. A. Adio.

REFERENCES

Adelusi, S. A., Salako, L. A. (1980) J. Pharm. Pharmacol. 32: 711–712

Brohult, J., Rombo, L., Sirleaf, V., Bengtsson, E. (1979) Ann. Trop. Med. Parasitol. 73: 401–405

Frisk-Holmberg, M., Bergkvist, Y., Domeij-Nyberg, B., Hellstrom, L., Jansson, F. (1979) Clin. Pharmacol. Ther. 25: 345–350

Fuld, H. (1959) Lancet 2: 617–618

Fuld, H., Horwish, L. (1958) Br. Med. J. 2: 1199–1201

Gaudette, L. E., Coatney, G. R. (1961) Am. J. Trop. Med. Hyg. 10: 321–326

Grundmann, M., Mikulikova, I., Vrublovsky, P. (1972) Arch. Int. Pharmacodyn. 197: 45–52

Laaksonen, A. L., Koskiahde, V., Juva, K. (1974) Scand J. Rheumatol. 3: 103–108

Lindquist, N. G. (1973) Acta Radiol. Suppl. 325: 1–92

McChesney, E. , Banks, W. F., Jr., Sullivan, D. J. (1965) Toxicol. Appl. Pharmacol. 7: 627–636

McChesney, E. W., Fasco, M. J., Banks, W. F., Jr. (1967) J. Pharmacol. Exp. Ther. 158: 323–331

McChesney, E. W., McAuliff, J. P. (1961) Antibiot. Chemother. 11: 800–810

Rubin, M., Bernstein, H. N., Zvaifler, N. J. (1963) Arch. Opthalmol. 70: 474–481

Rubin, M., Zvaifler, N. J., Bernstein, H. N., Mansour, A. (1965) Proc. 2nd Int. Cong. Pharmacol. 467–486, Pergamon Press, Oxford

Zvaifler, N. J., Rubin, M., Bernstein, H. N. (1963) Arthrit. Rheum. 6: 799–809