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Saliva secretion of chloroquine in man

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The presence of chloroquine in saliva from seven healthy volunteers for 21 days after a single 600 mg oral dose of the drug was established by chromatographic and spectroscopic methods. The results showed the drug to be rapidly absorbed with a t_{max} in saliva of 3·9 ± 1·6 h. A long elimination half-life of 7–20 days, calculated from the saliva concentration-time profile, is in agreement with values previously reported for the drug using plasma level data. In three of the volunteers from whom simultaneous blood samples were obtained, the mean C_s/C_p value was 1·20 ± 0·27. It is suggested that saliva level determination may be used in evaluating patient compliance, therapeutic drug monitoring and pharmacokinetic parameters of chloroquine.

Chloroquine is used both in the prophylaxis and treatment of malaria, and in treating rheumatoid arthritis and other collagen diseases for which it has a narrow therapeutic range (0·6–2·5 μmol litre^{-1} serum for chronic rheumatoid arthritis, Frisk-Holmberg et al 1979). It also gives rise to side effects when used in the prophylaxis and treatment of malaria, hence there is a need for its presence in the tissues to be monitored during long term therapy to avoid toxicity.

While blood is usually used for this purpose, measurement of saliva concentrations may reflect blood values and also have the advantages of convenience, painlessness and non-invasiveness and also of measuring the free form of the drug (Mucklow 1982).

Theoretically, the saliva/plasma ratio of basic drugs like chloroquine ($C_s : C_p$) can be predicted from equation 1 (Matin et al 1974).

$$\frac{C_s}{C_p} = \frac{1 + 10^{(pK_a - pH_s)}}{1 + 10^{(pK_a - pH_p)}} \times \frac{f_p}{f_s} \qquad (1)$$

pH_s = saliva pH, pH_p = plasma pH, f_p = the fraction of drug free (unbound) in plasma, f_s = the fraction of drug free in saliva.

Although it is known that the non-protein bound (free) form of chloroquine distributes widely throughout the body (Berliner et al 1948; Schaffer et al 1958; Kuroda 1962), no information has been published on its availability in human salivary secretions. Of all the antimalarials, only pyrimethamine has been detected and measured in human saliva (Ahmad & Rogers 1981).

We set out to demonstrate the presence of chloroquine in human saliva and to determine the chloroquine levels in this secretion after a single oral dose.

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Methods

Seven apparently healthy volunteers (five male, two female) aged between 23 and 28 years (25·7 ± 2·0) consented to participate in the study which was approved by the ethics committee of Ife University Teaching Hospitals Complex. Before drug administration, blank saliva and, in three volunteers, blank blood samples were collected. Each volunteer received a single 600 mg dose of chloroquine as 4 tablets of Ronaquine. The only other drug allowed was for one volunteer who took promethazine (Phenergan) 1 × 25 mg tablet, 30 min before the chloroquine tablets. Stimulated mixed saliva was obtained, after a wash of the mouth with water, by each volunteer sucking a sterilized glass bead and allowing saliva to accumulate until the desire to swallow, after which the fluid was expelled smoothly into a wide-mouthed bottle. A minimum of 3 ml of mixed saliva was collected over a 10 min period at 0, 1, 2, 3, 4, 6 and 24 h, then once daily to the 7th day, and thereafter once on alternate days until the 21st day after drug administration. In three volunteers simultaneous venous blood samples were taken up to the 3rd day into heparinized tubes by venepuncture of the antecubital vein. Blood samples were centrifuged immediately to obtain the plasma which was either analysed immediately or stored at −20 °C until analysed. All saliva samples were frozen for at least 4 h to allow accurate measurements of volumes for analysis.

An ion-pair HPLC method developed in our laboratory was used for the analysis. It involved the use of a C-18 reversed phase column with detection by UV at 254 nm. The mobile phase was methanol–0·2 M sodium dihydrogen phosphate (1 : 1) containing 75 mmol litre^{-1} perchloric acid.

For the TLC analysis, pooled blank and test saliva samples were separately extracted under alkaline (pH 12) conditions with n-hexane. The concentrated extracts were run on pre-coated fluorescent silica gel plates (Merck), 20 × 10 mm (0·25 mm thick) on which chloroquine was also spotted. The plates were then developed using two solvent systems viz. I, chloroform–ethylmethylketone–diethylamine (50 : 40 : 10) and II, toluene–methanol–diethylamine (75 : 15 : 10). The plates were viewed under a UV lamp (254 nm). The spot corresponding to chloroquine on the plate was scraped and extracted with n-hexane and back-extracted into the HPLC solvent system and the UV spectrum of this solution run on a recording spectrophotometer. A

solution of a known concentration of chloroquine in the same solvent system was similarly treated.

Results

The pooled test saliva extract gave two spots on the TLC plates with systems I and II and these gave orange colouration with Dragendorff's reagent. These spots were absent from the blank saliva extract run on the same plate. The reference chloroquine sample gave only one spot with the same R_F values of 0·64 and 0·42 in systems I and II, respectively, as one of the spots from the test saliva extract. The second spot on the plates had chromatographic characteristics similar to desethylchloroquine run on the same plates which had the R_F values of 0·52 and 0·32 in systems I and II, respectively. The chloroquine spot from the test extract and reference sample gave a purple colour under UV (254 nm). The UV spectroscopic analysis of the extract of the spot corresponding to chloroquine gave an identical and superimposable spectrum with chloroquine solution. The extract of the spot on HPLC gave a single peak corresponding to the retention time (Rt) of chloroquine of 6·5 min (Rt of internal standard, papaverine, 10·8 min). The other peak found in both saliva and plasma had the same Rt (5 min) as desethylchloroquine. Chloroquine could be detected in the saliva of all the volunteers throughout the 21 day period of sampling.

The time course of chloroquine concentrations in the saliva of one volunteer is shown in Fig. 1.

Some pharmacokinetic parameters obtained for chloroquine in saliva from the individual volunteers are shown in Table 1. The salivary terminal $t_{\frac{1}{2}}$ of the drug was calculated by linear regression analysis using at least 3 points in the terminal phase of the concentration–time curves. The $t_{\frac{1}{2}}$ varied from 7 to 20 days.

For those volunteers whose blood samples were collected, the ratio of salivary concentration (C_s) to plasma concentration (C_p) were calculated for each sample time. The concentration of chloroquine in saliva was found to be higher than in plasma from samples taken at the same time. The C_s/C_p values in the three volunteers were 0·44–3·08, 0·64–1·38 and 0·62–1·45 respectively. The mean value was 1·20 ± 0·27.

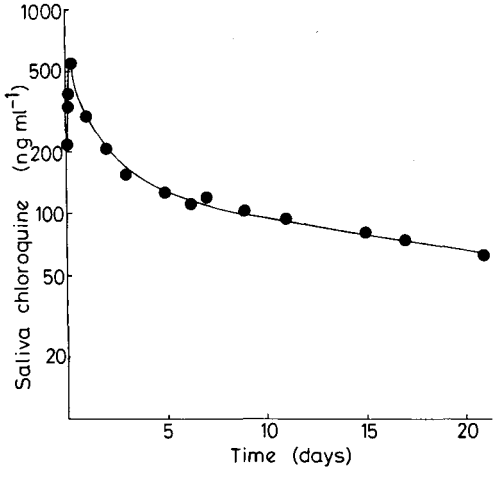

FIG. 1. Saliva concentration–time curve of chloroquine obtained from a volunteer after a single 600 mg oral dose of chloroquine.

Table 1. Some pharmacokinetic parameters of chloroquine derived from the salivary concentration data after a single 600 mg oral dose.

Volunteer	C^s_{max} (ng ml^{-1})	t^s_{max} (h)	$t_{\frac{1}{2}}$ (day)
I	292·8	3	16·1
II	1092·0	3	7·2
III	1151·6	4	8·9
IV	261·8	3	10·9
V	924·0	2	12·2
VI	551·9	6	16·2
VII	1005·2	6	19·5
Mean	754·2	3·9	13·0
with s.d.	378·6	1·6	4·4

Discussion

The t_{max} of chloroquine of 2 to 6 h (3·9 ± 1·6 h) in saliva obtained in this study is similar to the literature values of 1 to 6 h (3·6 ± 2·0 h, n = 11) found by Gustafsson et al (1983). The peak concentration (C_{max}) occurred at about the same time in saliva and in plasma and suggests that equilibrium is reached rapidly between them, in line with Posti's hypothesis that saliva should be regarded as an integral part of the central compartment (Posti 1982) rather than a 'deep' pharmacokinetic compartment as suggested by Galeazzi et al (1976). This is confirmed by the fact that the $t_{\frac{1}{2}}$ of 7 to 20 days we obtained falls within the range previously reported for the drug from plasma level data. For example, Gustafsson et al (1983) reported terminal elimination $t_{\frac{1}{2}}$ values ranging from 7–14 days after a single dose of the drug. A recent review by White (1985) put the $t_{\frac{1}{2}}$ range at 6–50 days. The profile of the drug in saliva (Fig. 1) is also similar to the multiexponential curves previously described by other workers using plasma level data.

The considerable intra- and inter-individual variation in the C_s/C_p ratio found is probably a consequence of the effect of salivary pH fluctuation in the subjects. Using equation 1 and the reported pK_a value of 10·8 (Pharm. Codex 1979), f_p of 39% (Walker et al 1983) and assuming $f_s = 1$, the theoretical C_s/C_p ratios for chloroquine using the two extreme saliva pH values of 6·5 and 7·2, were 3·10 and 0·62, respectively. The C_s/C_p ratio obtained in this work varied within these theoretical values.

In conclusion, our results suggest that saliva level determination may prove of use in the assessment of patient compliance with chloroquine therapy as well as in therapeutic drug monitoring and determination of some pharmacokinetic parameters of the drug.

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REFERENCES

Ahmad, R. A., Rogers, H. J. (1981) Br. J. Clin. Pharmacol. 11: 101–102

Berliner, R. W., Earle, D. P., Taggart, J. V., Zubrod, C. G., Welch, W. J., Conan, N. J., Banman, E., Scudder, S. T., Shannon, J. A. (1948) J. Clin. Invest. 27 Suppl: 98–107

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

Galeazzi, R. L., Benet, L. Z., Sheiner, L. B. (1976) Ibid. 20: 278–289

Gustafsson, L. L., Walker, O., Alvan, G., Beerman, B., Estevez, F., Gleisner, L., Lindström, B., Sjoqvist, F. (1983) Br. J. Clin. Pharmacol. 15: 471–479

Kuroda, K. (1962) J. Pharmacol. Exp. Ther. 137: 156–161

Matin, S. B., Wan, S. H., Karam, J. H. (1974) Clin. Pharmacol. Ther. 16: 1052–1058

Mucklow, J. C. (1982) Ther. Drug Monit. 4: 229–247

Pharmaceutical Codex (1979) 11th Edn. The Pharmaceutical Press, London, p. 176

Posti, J. (1982) Pharm. Acta Helv. 57: 83–92

Schaffer, B., Cahn, M. M., Levy, E. J. (1958) J. Invest. Derm. 30: 341–345

Walker, O., Birkett, D. J., Alvan, G., Gustafsson, L. L., Sjoqvist, F. (1983) Br. J. Clin. Pharmacol. 15: 375–377

White, N. J. (1985) Clin. Pharmacokinet. 10: 187–215

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The influence of chronic or acute nicotine pretreatment on ethanol-induced gastric ulceration in the rat

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The effects in rats of chronic or acute nicotine pretreatment were studied on three gastric parameters: ethanol-induced ulceration, gastric wall mucus content and gastric acid secretion, under basal or histamine-stimulated conditions. Oral administration of ethanol (40%, 10 ml kg^{-1}) depleted gastric wall mucus and produced ulceration in the gastric glandular mucosa. Ten-day nicotine pretreatment (15 or 25 μg ml^{-1} drinking water) worsened the adverse effects of ethanol on mucosal ulceration and mucus content, potentiated the gastric secretory action of histamine, but did not affect basal acid secretion. Single oral doses of nicotine (2 or 4 mg kg^{-1}, given 1 h beforehand) prevented ulceration and mucus depletion in ethanol-treated animals; however, they did not influence either basal or histamine-stimulated gastric acid output. It is concluded that chronic nicotine administration aggravates ethanol ulceration, possibly by decreasing gastric wall mucus content and sensitizing the stomach to the acid secretory action of histamine. On the other hand, an acute oral dose of nicotine preserves the mucus content and prevents ethanol-induced ulcer formation.

Chronic nicotine pretreatment has been found to worsen ethanol-induced ulceration in the gastric glandular mucosa of rats (Ogle et al 1985). The association between peptic ulcers and cigarette smoking has been shown in man (Friedman et al 1974), but the mechanism for the aggravation of ethanol-induced ulceration by chronic nicotine administration in rats is yet to be defined.

It is known that gastric wall mucus plays an important role in the defensive mechanism of the stomach against acid-pepsin digestion (Bickel & Kauffman 1981; Pfeiffer 1981; Williams & Turnberg 1980), and that excessive gastric histamine leakage appears to contribute largely to ethanol ulceration (Cho et al 1983; Dinoso et al 1976). The present study examines the influence of chronic or acute nicotine pretreatment on gastric ulceration and mucus content, both in the absence and presence of ethanol, and on the acid-secretory action of histamine in rat stomachs.

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Methods

Male Sprague-Dawley rats (180–210 g) were reared on a standard laboratory diet (Ralston Purina Co.) and given tap water to drink. They were kept in a temperature (22 ± 1 °C)- and humidity (65–70%)-controlled room where the experiments were conducted.

In the chronic nicotine experiments, rats drank either ordinary tap water or nicotine bitartrate (BDH), 5 or 25 μg ml^{-1} of tap water, for 10 days (each rat drank 31 ± 1·8 ml per day, i.e. 155 ± 9 or 775 ± 45 μg nicotine per day); the weight of the alkaloid is expressed as its salt. Food, but not drinking fluid, was removed on the 9th day. On the 10th day, rats were given either distilled water or 40% v/v ethanol (BDH) in distilled water, in a volume of 10 ml kg^{-1}, orally via a stainless steel gastric tube. All animals were killed by a sharp blow on the head 5 h later. Stomachs were removed, opened along