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Excretion of proguanil in human saliva

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Abstract—After a single oral administration of a 300 mg dose of proguanil to six volunteers, the presence of the drug in saliva was established by chromatographic and spectrophotometric methods. The t_{max} and elimination half-life of proguanil derived from salivary levels were $4{\cdot}0 \pm 1{\cdot}26$ h and $15{\cdot}1 \pm 1{\cdot}8$ h, respectively. These results are in agreement with values previously reported for the drug using plasma level data. The mean saliva: plasma proguanil concentration ratio was $0{\cdot}41 \pm 0{\cdot}17$ and this was not time dependent. There was a correlation ($r = 0{\cdot}82$) between the saliva and simultaneous plasma proguanil concentration. The results suggest that proguanil is passively secreted into saliva and that saliva levels may be useful in the determination of pharmacokinetic parameters and the therapeutic monitoring of the drug.

Proguanil is widely used in Nigeria as a prophylactic agent against malaria infection and is a drug of choice for malaria suppression in sickle-cell patients, particularly children. It is administered chronically on a daily basis. As it has been asserted that the pharmacokinetics of some drugs may be altered during long term use (Bousquet 1970) monitoring of drug levels in the body is necessary. With proguanil this is necessary as it occasionally fails to provide the prophylactic effect despite strict adherence to the recommended dosage regimen (WHO 1965). For some drugs, their monitoring of salivary concentration offers advantages over blood sampling (Mucklow 1982). Two antimalarial drugs previously demonstrated to be secreted in human saliva are pyrimethamine (Ahmad & Rogers 1981) and chloroquine (Ogunbona et al 1986). We set out to establish the presence of proguanil in human saliva, evaluate the salivary pharmacokinetics of the drug and investigate the correlation between saliva and plasma levels of the drug after a single oral dose.

Material and methods

Six apparently healthy volunteers, five male, one female, 20–24 years, 53 to 70 kg, took part after giving their informed written consent. The study was carried out with the approval of the Ethics Committee of the Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria. The volunteers took no drug for at least two weeks before the study and were not allowed to take any other drug throughout the duration of the study. Before administration of proguanil, blank saliva and blood samples were collected. Each volunteer received a single 300 mg dose of proguanil as three tablets of Paludrine. After a wash of the mouth with water, stimulated mixed saliva was collected by each volunteer sucking a sterilized glass bead and allowing the saliva to accumulate before expelling the fluid into a glass jar. A minimum of 5 mL of mixed saliva was collected over 5 min at 0·5, 1, 2, 3, 4, 5, 7, 12, 24, 36 and 48 h after drug administration. Also in all the volunteers, blood samples were taken simultaneously at 3 h, 5 h and 24 h after drug administration, by venipuncture of the antecubital vein. The blood samples were centrifuged immediately for 10 min at 2000 *g* to obtain the plasma. The pH of the saliva samples was taken as soon as possible and the samples were frozen for at least 4 h to allow accurate measurement of volume for analysis. All samples were stored at −20°C when not immediately analysed.

The concentrations of proguanil in the plasma and saliva samples were determined using a liquid chromatographic method developed in our laboratory. The drug and the internal standard (pyrimethamine) were extracted with ether at pH 12. The organic phase was transferred into a tapered-end tube and back-extracted with 100 μL 0·1 M HCl. The organic phase was rejected and 20 μL of the aqueous extract was then chromatographed on a C_{18} reversed phase column (5 μm) through which is pumped at 1 mL min^{-1} a solvent system of methanol *en* 0·5% ammonium acetate (1:1) containing 50 mM perchloric acid. The detection was by UV at 254 nm. The limit of detection of proguanil was 12 ng mL^{-1}.

The TLC analysis was carried out by separately extracting pooled blank and test saliva samples with benzene under alkaline conditions (pH 13). The extracts were concentrated under vacuum at 50°C on a rotary evaporator. A TLC of the concentrated extracts was run on pre-coated fluorescent silica gel plates (Merck). Proguanil solution in benzene was also spotted on the same plates to serve as reference. The plates were developed using three solvent systems viz: I, Toluene–methanol–diethylamine. (75:15:10) II, methanol–ethylmethylketone–diethylamine (45:45:10) and III, glacial acetic acid–water (40:60). The plates were viewed under a UV lamp (254 nm) for detection of the spots. The spot corresponding to proguanil was scraped and extracted with benzene, back-extracted into 0·1 M HCl and injected onto the HPLC. The UV spectrum of this aqueous layer was also run on a recording spectrophotometer. A blank spot on the plate was scraped and similarly extracted. The aqueous layer served as the blank in the UV analysis. The UV spectrum of a 5 μg mL^{-1} solution of proguanil in 0·1 M HCl was also recorded.

Results

The pooled blank saliva extract showed no spot on TLC whereas the pooled test saliva extract had one spot that gave a purple colour in UV (254 nm) and orange colouration with Dragendorff's reagent. The reference proguanil behaved in the same way. The R_F values of the spot and proguanil were the same in the three systems: system I 0·35, system II 0·61 and system III 0·89. The UV spectrum of the extract of the spot was identical with the UV spectrum of the proguanil solution with absorption maximum of 247 nm. The extract of the spot also gave a single peak ($Rt = 12{\cdot}5$ min) when injected onto the HPLC. This corresponds to the Rt of proguanil. The Rt of the internal standard was 9·0 min.

Table 1 shows the pharmacokinetic parameters of proguanil derived from the saliva concentration data of each of the volunteers. The elimination half-life ($t_{\frac{1}{2}}$) of the drug was calculated by linear regression analysis using at least three points in the terminal phase of the concentration-time profiles. Table 1 also shows that although there were inter- and intra individual variation in the saliva to plasma proguanil concentration ratio (Cs/Cp), the mean Cs/Cp values monitored at the 3rd, 5th and 24th h after administration of the drug, showed no significant difference ($P > 0{\cdot}1$) from each other. Overall, the mean Cs/Cp

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Table 1. Some pharmacokinetic parameters of proguanil derived from the salivary concentration data and the saliva to plasma proguanil concentration ratio obtained after single 300 mg oral dose of proguanil.

Volunteer	t_{max} (h)	C^s_{max} ng mL^{-1}	$t_{\frac{1}{2}}$ (h)	Cs/Cp at 3 h	5 h	24 h
I	3	167·7	15·8	0·56	0·19	0·30
II	5	140·0	11·4	0·33	0·50	0·27
III	4	148·6	16·5	0·27	0·23	0·44
IV	2	455·5	15·1	0·57	0·56	0·21
V	5	95·1	16·9	0·21	0·36	0·37
VI	5	244·7	15·8	0·62	0·64	0·74
Mean	4·0	208·6	15·1	0·43	0·41	0·39
±s.d.	1·26	130·5	1·87	0·17	0·18	0·19

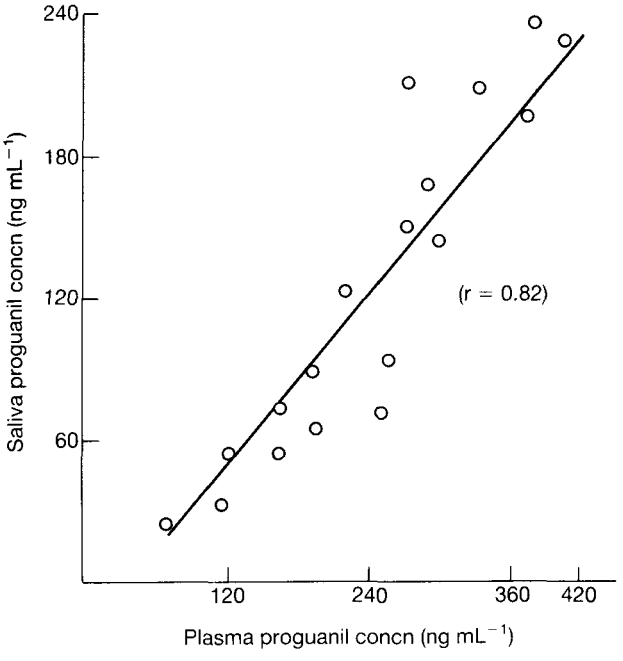

FIG. 1. A plot of saliva proguanil concentration against simultaneous plasma levels monitored after a single 300 mg oral administration of proguanil.

was 0·41 ± 0·17. The saliva pH ranged from 7·0 to 7·3 with a mean of 7·2 ± 0·15.

There was a correlation (r) of 0·82 between the saliva and simultaneous plasma levels of proguanil (Fig. 1).

Discussion

The t_{max} and elimination half life ($t_{\frac{1}{2}}$) of proguanil monitored in the saliva were in the range of previously reported values derived from plasma data. For example, the t_{max} obtained in this work was 4·0 ± 1·26 h which is comparable to the t_{max} of about 4 h reported by Ritschell et al (1978) and White (1985). The saliva proguanil $t_{\frac{1}{2}}$ which ranged from 11·4 to 16·9 h (15·1 ± 1·87) are in agreement with the literature values of about 15 h (Ritschell et al 1978) generated from plasma concentration profiles.

It has been asserted that saliva pH is the major variable determining the concentration of ionizable drugs in saliva. Also, the appearance in saliva of basic drugs with a pKa over 5·5 is profoundly affected by minor alterations in salivary flow rate and thus pH (Mucklow et al 1978). Since the pKa of proguanil is 10·4 (Clarke 1971), its salivary excretion would be expected to be affected by the pH of saliva. However, a correlation was observed to exist between the saliva and plasma levels of the drug. This is most probably due to the narrow range (7·0 to 7·3) of pH values of saliva obtained in this work. This is a reflection of the advantage of collection of stimulated saliva which has a narrow range around the value of 7·0 (Gilbaldi & Prescott 1983), whereas the pH of unstimulated saliva showed a larger variability.

The Cs/Cp values obtained were all less than unity. This might not be unexpected because 75% of the plasma concentration of proguanil is bound to plasma protein (Ritschell et al 1978). Hence, at any point in time, only about 25% of the plasma drug concentration will be free (unbound form) to traverse the lipoidal membrane of the salivary gland. Assuming proguanil were passively transferred to saliva, the mean theoretical value of the Cs/Cp ratio of the drug could be predicted by inserting the value of pKa 10·4, pHp 7·4, pHs 7·2, fp 0·25 into the equation of Matin et al (1974) and assuming that fs = 1. The theoretical Cs/Cp ratio of 0·40 is obtained and this is in complete agreement with the mean values of the same ratio obtained in this study. This suggests that proguanil is probably passively transferred into saliva.

In conclusion, the significance of the findings here is that saliva level determination can be substituted for blood level determination in therapeutic drug monitoring and evaluation of pharmacokinetic data of proguanil in man.

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