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Salivary secretion of paracetamol in man

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Plasma and saliva paracetamol levels were measured by HPLC in ten healthy volunteers who took a therapeutic dose after an overnight fast. Salivary levels of the drug were consistently and significantly higher than those in plasma for the first 50 min after oral ingestion, but saliva and plasma levels correlated closely during the elimination phase. There was a highly significant correlation between the AUC 0-α calculated from saliva and plasma paracetamol concentration-time curves. The elevated saliva/plasma ratio for the first 50 min was not due to loss of paracetamol from plasma during sample preparation, binding to plasma protein or adsorption to the buccal mucosa. Administration of probenecid in an attempt to block possible active secretion of paracetamol into saliva did not significantly alter the saliva/plasma concentration ratio for the first 50 min, but did significantly reduce this ratio in the time period 125–360 min. The experimental data conformed to a recently proposed model in which elevated saliva/plasma ratios during the early stages following oral ingestion were related to ongoing absorption into the arterial system.

The concentration of drugs in saliva and plasma often correlate closely (Graham & Rowland 1972; Matin et al 1974; Reynolds et al 1976), with plasma levels tending to be higher. However, during studies of paracetamol metabolism we noticed that higher values tended to be obtained in saliva than in plasma for up to 2 h after an oral dosing. These findings agreed with those reported by Glynn & Bastain (1973) and Adithan & Thangam (1982), but no mechanism was suggested by those authors to account for this phenomenon. We have therefore sought to determine if factors such as paracetamol protein binding, sample preparation method, active transport process and/or the ongoing absorption process were responsible for such an effect. This report presents the finding of these studies.

Methods

Ten healthy volunteers (5 males, age 20–27 years, 56·5–83·5 kg) who had fasted overnight took an oral therapeutic dose of paracetamol (3 × 500 mg tablets, BP formulation) washed down with water (200 mL). Immediately afterwards they rinsed their mouths with a wash of 10% (v/v) ethanol solution (approx. 200 mL) to remove any traces of the drug. Saliva samples were obtained at 0, 15, 25, 35, 50, 65, 95, 125, 190, 240, 300, and 360 min after ingestion by direct spitting into plastic bottles, salivary flow having been stimulated by chewing on a piece of parafilm (50 mm × 50 mm approx.) for 2 min. Blood samples were taken simultaneously from an indwelling needle into heparinized tubes. This protocol was carried out on two occasions, separated by at least one week, once, at random, 1 h after an oral administration of probenecid (2 × 500 mg Benemid tablets). Ethical approval for these studies was obtained from the Medical School Ethical Committee.

Plasma and saliva samples were stored at −20 °C before measurement of paracetamol concentrations by the HPLC method of Howie et al (1977). Before analysis an equal volume of 25% (w/w) trichloroacetic acid (TCA) was added to each of the plasma and saliva samples which, after mixing, were centrifuged at 4000*g* for 10 min and 10 000*g* for 20 min, respectively; the resulting supernatants were used.

To determine if the use of TCA as a protein precipitant affected the recovery of paracetamol, plasma and saliva samples were obtained from five healthy volunteers, and these samples, and others of distilled water, were spiked with known amounts of paracetamol, so that the final concentration was 30 $\mu g\ mL^{-1}$. These samples were then incubated at 37 °C for 1 h in a shaking bath, followed by deproteinization with TCA and determination of paracetamol levels. To determine the extent of paracetamol protein binding, plasma and saliva samples spiked with paracetamol (concentration of 30 $\mu g\ mL^{-1}$) were equilibrated with an equal volume of 0·1 M phosphate buffer, pH 7·4 in specially constructed dialysis cells at 37 °C for 3 h. After this time, the concentration of paracetamol in the buffer solution was measured. To determine if paracetamol was adsorbed onto the buccal tissue, 3 × 500 mg tablets were crushed and suspended in 200 mL of water. A mouthful of the suspension was retained in the mouth by a subject for 1 min, and then discarded. Immediately afterwards the mouth was rinsed and saliva samples were taken at 0, 15, 25, 35 and 50 min. At a later date, three paracetamol tablets (1·5 g) were ingested with 200 mL water by the same subject and immediately afterwards the mouth was rinsed and one saliva sample was collected and analysed.

Saliva and plasma paracetamol half-lives were calculated by the method of least squares analysis. Area under the curve (AUC) was calculated by the trapezoidal rule, and extrapolation to infinity. Statistical analysis was carried out by a paired sample Student's *t*-test or analysis of variance (using SPSS-X package) as appropriate. Values reported in the text are given as mean ± s.e.m.

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Results

Paracetamol was detectable in plasma and saliva at 15 min after oral ingestion of the drug (Fig. 1a) and thereafter the concentration-time curves had similar patterns. Significantly higher concentrations of paracetamol in saliva relative to those in plasma were observed during the first 50 min following oral ingestion ($P < 0.001$, analysis of variance). There was a good correlation between plasma and saliva paracetamol levels (70 sample pairs) during the latter, 65–360 min, phase of the

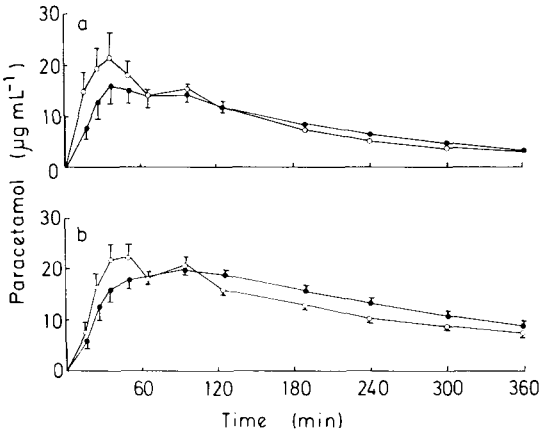

FIG. 1. Saliva (○) and plasma (●) paracetamol concentrations at various times after oral ingestion of 1·5 g paracetamol, (a) without and (b) with pre-treatment with probenecid. Values are mean ± s.e.m. of 10 subjects.

experiment ($r = 0.895$, $P < 0.001$). The elimination half-lives calculated from plasma and saliva data for each subject are indicated in Table 1. These values were similar within each subject, and the mean saliva and plasma elimination half-lives were found not to be significantly different (saliva: 2·0 ± 0·1 h; plasma 2·2 ± 0·2 h; $P > 0.05$, *t*-test). There was a highly significant correlation ($r = 0.951$, $P < 0.001$) between the AUC 0-α calculated from the saliva and plasma paracetamol cocnentration-time curves. There were no significant differences between males and females in the saliva to plasma concentration ratio at any of the experimental time-points.

Table 1. Elimination half-lives of paracetamol for 10 subjects calculated from plasma and saliva samples. The data obtained from the studies with no probenecid and after probenecid treatment are presented.

	Elimination half-life (h)			
	No probenecid		After probenecid	
Subject	Plasma	Saliva	Plasma	Saliva
1	2·8	2·5	3·8	4·6
2	1·6	1·7	2·7	2·8
3	2·3	1·8	4·0	4·4
4	2·2	2·5	3·3	3·3
5	1·8	1·8	3·3	3·8
6	1·9	2·1	4·0	4·3
7	3·1	2·4	3·6	2·9
8	2·3	2·0	4·0	3·8
9	1·8	1·9	2·7	2·8
10	1·8	1·3	2·8	2·6

Treatment of spiked saliva and plasma samples with TCA produced virtually equivalent losses of paracetamol (13·7 ± 7·8% and 11·3 ± 7·5%, respectively). Paracetamol was not bound to protein in saliva but was bound to protein in plasma to the extent of 9·6 ± 2·4%. Although paracetamol (up to 15 μg mL^{-1}) could be detected in saliva during the 50 min after a suspension had been held in the mouth for 1 min, no detectable amounts were measured when mouth washing immediately followed drug ingestion (experimental limit of paracetamol detection being 0·1 μg mL^{-1}).

In an attempt to block possible secretion of paracetamol into saliva by active transport, probenecid was given 1 h before ingestion of paracetamol at a dose which effectively blocked active secretion of paracetamol sulphate in the kidney (Fry & Kamali 1985a). The concentration of paracetamol in saliva was significantly greater than that in plasma during the first 50 min after ingestion of paracetamol (Fig. 1b; $P < 0.001$, analysis of variance), and the pre-treatment with probenecid did not significantly alter either the saliva or plasma paracetamol levels during this time, when compared with those obtained with no pretreatment. The concentrations in saliva were significantly lower than those in plasma in the time interval 125–360 min (Fig. 1b; $P < 0.001$, analysis of variance). The elimination half-life of paracetamol measured in plasma or saliva was prolonged in the presence of probenecid, there being no significant difference between the values for saliva and plasma (saliva: 3·5 ± 0·2 h; plasma 3·4 ± 0·2 h; $P > 0.30$, *t*-test). There was no significant difference in the rate or extent of absorption of paracetamol in the presence of probenecid as judged by calculation of the time to peak plasma levels and the plasma AUC 0–95 (data not shown).

Discussion

Significantly higher levels of paracetamol were detected in saliva compared with plasma for the first 50 min following oral ingestion of the drug, the levels thereafter being comparable. The finding is in agreement with the data of Glynn & Bastain (1973) and Adithan & Thangam (1982), although in neither study did the authors carry out any appropriate statistical analysis or suggest a possible mechanism for this phenomenon. The finding of essentially identical saliva and plasma paracetamol elimination half-lives is also in agreement with the data of Adithan & Thangam (1982).

Matin et al (1974) have suggested that for a lipid soluble acidic compound such as paracetamol (pK_a = 9·5) the saliva to plasma concentration ratio (R) could

be predicted on the basis of the degree of ionization in the two fluids by the following equation:

$$R = \frac{1 + 10^{(pH_s - pK_a)}}{1 + 10^{(pH_p - pK_a)}}$$

where pH_s = pH of saliva and pH_p = pH of plasma. If total drug concentration (i.e. bound + unbound) is measured, then the ratio would be predicted by a derived equation:

$$R = \frac{1 + 10^{(pH_s - pK_a)}}{1 + 10^{(pH_p - pK_a)}} \times \frac{F_p}{F_s}$$

where F_p = fraction of unbound drug in plasma, and F_s = fraction of unbound drug in saliva.

We have determined that there is no selective loss of paracetamol from plasma samples during preparation for analysis, and that paracetamol is not bound to saliva protein and only minimally bound to plasma protein. We have also determined that, under the conditions of the experiments, paracetamol is not adsorbed onto the buccal mucosa. Also, under conditions of stimulated salivary flow, as was achieved in our experiments, the pH of saliva approaches that of plasma (Dawes & Jenkins 1964). Because of this, it would be expected from the equations of Matin et al (1974) that the saliva to plasma concentration ratio for paracetamol would aproach unity, and, thus, the significantly higher levels of paracetamol in saliva relative to those in plasma observed in the first 50 min are at variance with the expected findings.

Whilst the passage of most drugs into saliva occurs by passive diffusion, it has been suggested that an active transport process may operate for some drugs (Borzelleca & Cherrick 1965; Galeazzi et al 1976; Dawes et al 1978: Boobis & Trembath 1978). Pretreatment with probenecid, at a dose which significantly inhibited the renal secretion of paracetamol sulphate, did not attenuate the elevated saliva levels of paracetamol relative to those in plasma during the first 50 min. This pretreatment did, however, lead to significantly lower saliva levels relative to those of plasma during the time period 125–360 min, thus suggesting that whilst secretion of paracetamol into saliva may involve an active transport component this cannot explain the apparently anomalous findings noted previously. Pretreatment with probenecid was associated with a prolongation of the elimination half-life of paracetamol, this being due to inhibition of paracetamol metabolism (Fry & Kamali 1985a, b).

Recently, Posti (1982) has developed an anatomical–physiological model to explain the finding of higher saliva drug levels relative to plasma drug levels during the early stages following oral ingestion of a drug. In this model it is hypothesized that: (1) during absorption the concentration of drug in arterial blood is higher than that in venous blood, this concentration difference being at any moment directly proportional to the rate of absorption, and (2) the concentration of drug in saliva is in equilibrium with that in arterial blood. The duration of the elevated saliva to plasma concentration ratio for paracetamol is consistent with the suggestion that the elevated ratio will occur during absorption, and the forms of saliva/plasma paracetamol concentration-time curves are in agreement with the general form predicted by Posti, after allowance for the possible active secretion process (Fig. 1b). One conclusion derived by Posti is that the difference in AUC between the saliva and plasma drug concentration-time curves within appropriate time limits during absorption is proportional to the amount of drug present in the system at time t2. To test the appropriateness of the Posti model to the present findings with paracetamol we have performed a correlation analysis on the AUC 0–50 vs (saliva concentration at 50 min) data, and obtained a significant correlation ($r = 0{\cdot}72$, $P < 0{\cdot}05$). It is therefore suggested that the elevated saliva to plasma concentration ratio for paracetamol observed during the first 50 min following oral ingestion is related to ongoing absorption of paracetamol into the arterial system.

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