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Mandibular and parotid salivary excretion of procainamide and *N*-acetylprocainamide after intravenous administration of procainamide to rats

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Flow rate, protein level and pH of mandibular and parotid saliva samples in rats were almost stabilized 2 h after induction of salivation with pilocarpine (9·0 mg h^{-1} kg^{-1}). Salivary excretion profiles of procainamide, 50 mg kg^{-1} i.v., and its metabolite *N*-acetylprocainamide (NAPA) were then investigated. Plasma and salivary procainamide levels declined bi-exponentially with time almost in parallel. Salivary procainamide levels (Y) from both types of gland were correlated with the plasma level (X) over a wide concentration range [$Y = 0{\cdot}108X + 1{\cdot}96$ ($r = 0{\cdot}795$) for mandibular and $Y = 0{\cdot}917X$ ($r = 0{\cdot}974$) for parotid glands]. The mean saliva to plasma concentration ratio (S/P ratio) was significantly higher in parotid (0·974 ± 0·243) than in mandibular (0·284 ± 0·119) saliva samples. Similar correlations and S/P ratios were observed for NAPA. Procainamide and NAPA mean S/P ratios for saliva from both glands were fairly consistent with the calculated value according to the pH-partition hypothesis.

It has been suggested that saliva samples might be substituted for plasma in therapeutic drug monitoring or some clinical pharmacokinetic studies if there is a consistent correlation of salivary and plasma drug levels over a wide range of concentrations of a drug (Dvorchik & Vesell 1976; Horning et al 1977). Furthermore, it has been reported that lipid solubility may be a determining factor in the salivary excretion of most compounds by a simple diffusion process (Rasmussen 1964; Gruneisen & Witzgall 1974). Matin et al (1974) have proposed that the saliva to plasma concentration ratio (S/P ratio) for weakly acidic or basic compounds can be predicted from a modified pH-partition hypothesis. However, relatively large variations in the S/P ratio for several drugs, including procainamide (Danhof & Breimer 1978) have limited the clinical use of saliva in monitoring drug levels. Previous reports have demonstrated the effects of gland type (parotid and mandibular) differences in salivary drug-protein binding, flow rate or pH on the S/P ratio and/or on the salivary clearance of various compounds in dogs (Watanabe et al 1981a, 1984, 1985a, b, 1986) and rabbits (Watanabe et al 1985c) and salivary excretion mechanism has been shown to modify the S/P ratio by the above key factors. Few experiments have been carried out in rats, however, since pharmacokinetic evaluation has been almost impossible due to the limited numbers of pooled saliva samples (Piraino et al 1976; DiGregorio et al 1977; Piraino et al 1980; Gans et al 1980).

The present study was designed to establish the experimental method for the periodical collection of mandibular and parotid saliva samples in rats, to examine the salivary excretion kinetics of procainamide and its metabolite *N*-acetylprocainamide after the intravenous injection of procainamide, to verify Matin's equation and to see if there are specific differences between glands in the S/P ratio.

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MATERIALS AND METHODS

Materials

Both procainamide hydrochloride and *N*-acetylprocainamide (NAPA) were donated by Daiichi Pharmaceutical Co. (Tokyo, Japan). All other chemicals and reagents including pilocarpine hydrochloride (Hoei Yakko Co., Osaka, Japan) for salivation, benzene (Dortite Luminazol, Wako Pure Chemicals Co., Nagoya, Japan) for extraction of procainamide and NAPA, were of analytical grade. Seamless cellulose tubing (type 8/32, diameter 6 mm, length 10 cm, Visking Co., Tokyo, Japan) was used for the equilibrium dialysis experiments, KN-201 constant rate infusion pump (Natsume Seisakusho Co., Tokyo, Japan) for the salivation with pilocarpine, a micro combined electrode GS-195C (Toa-Dempa Co., Nagoya, Japan) for determination of salivary

pH and a RF-510 spectrofluorometer (Shimadzu Seisakusho Co., Kyoto, Japan) for the analysis of drugs in plasma and saliva samples.

Animals

Male Wistar rats (390–450 g, 16- to 18-week-old) purchased from Shizuoka Laboratory Animal Farm (Hamamatsu, Japan) were anaesthetized with pentobarbitone (50 mg kg^{-1} i.p.) after overnight fasting for 15 h. Body temperature was kept at 37·5 °C by means of thermostatic heating pads placed under the rats.

Collection of saliva samples

After tracheotomy and catheterization, cannulae were made to collect mandibular and parotid saliva samples separately by slight modification of the methods of Abe et al (1977) and Yoshida et al (1967). Bevelled PE-10 tubing (o.d. 0·61 mm, i.d. 0·28 mm, about 6 cm, Clay Adams, Tokyo, Japan) was inserted (2 to 5 mm in depth) into one of the paired mandibular duct orifices at the tongue base in the submandibular space and into one of the paired parotid duct orifices located on both sides of the buccal cavity. Both cannulae were sufficiently stable for periodical saliva collection over 6 h. For continuous salivation (over 6 h), pilocarpine hydrochloride solution in 0·9% NaCl (saline) was infused at 9 mg (base) h^{-1} kg^{-1} via the femoral vein cannula (PE-50). Salivary pH was measured immediately after collection of the sample by the method of Watanabe et al (1981a, b, 1984, 1985a, b, c, 1986). Salivary flow (i.e. volume min^{-1}) was estimated from the sample weight assuming that its specific gravity was 1·00 (Watanabe et al 1981a). Salivary protein level was determined by Lowry's method (1951) with bovine plasma albumin (Fraction V, Armour Pharmaceutical Co., purchased from Sanko Pure Chemicals Co., Tokyo, Japan) as standard. Pooled saliva samples up to the first 2 h were used as the blank saliva.

Plasma and saliva concentrations of procainamide and NAPA after intravenous injection of procainamide

On reaching steady-state salivation (in the flow, pH and protein level) following constant rate infusion of pilocarpine for 2 h, procainamide was administered (50 mg kg^{-1} as base) as a bolus dose via the jugular vein cannula as reported by Iwamoto et al (1982). Saliva samples were collected at various times for 4 to 10 min, and a blood sample (0·25 mL) was obtained at the midpoint of each saliva collection interval. Immediately after measuring the pH, the saliva samples less than 0·05 mL (parotid) or 0·1 mL (mandibular) were diluted by adding appropriate amount of the blank saliva.

Procainamide or NAPA in plasma (0·1 mL) and mandibular (0·1 mL) and parotid (0·05 mL) saliva, was determined by a modification of the method of Matusik & Gibson (1975). 0·2 mL of 1 M NaOH and 0·2 g of NaCl were added to the diluted (1- to 4-fold) sample and the mixture was extracted with 5 mL of benzene by vigorous shaking for 15 min. The separated benzene layer (4 mL) was re-extracted with 1 to 4 mL of 0·1 M HCl by shaking. The resultant aqueous layer (pH 1·0) was used for fluorometric analysis for NAPA at an excitation wavelength (λ_{ex}) of 270 nm and at an emission wavelength (λ_{em}) of 340 nm. 0·6 to 3 mL of this acidic solution was then made alkaline by adding 0·025 or 0·05 mL of 10 M NaOH. The fluorescence of this aqueous phase was measured for procainamide at λ_{ex} of 275 nm and at λ_{em} of 375 nm. The existence of unchanged drug in each acidic extract (for plasma and saliva) did not affect the determination of NAPA, and the existence of NAPA in both saliva samples did not affect the determination of procainamide. However, the calibrated value for procainamide in plasma was corrected by taking the NAPA concentration into account.

Equilibrium dialysis

0·5 mL of the plasma sample containing 8 or 20 μg mL^{-1} procainamide was dialysed against 2 mL of isotonic phosphate buffer solution (pH 7·4) using the seamless cellulose tubing at 37 °C. Its concentration in both phases after the equilibrium for 12 h was determined as described above.

Pharmacokinetic analysis and statistics

The procainamide concentration-time curve for plasma and saliva samples was analysed according to the least-squares regression program for two-compartment open model (FITTING CASE 3-1,2) by weighting with unity. Significant difference was determined by Student's *t*-test.

RESULTS

Salivary flow, protein level and pH during pilocarpine infusion

During intravenous pilocarpine infusion (9·0 mg h^{-1} kg^{-1}) over 6 h, mandibular saliva flow declined in the first 30 min and recovered gradually thereafter, whereas the parotid flow decreased slowly in the first 60 min. The mandibular flow was always the greater. The mean ± s.d. of the flow rate

in the first 4 h of sampling was 28·3 ± 15·8 (n = 75) and 11·06 ± 6·5 (n = 92) μL min^{-1} kg^{-1} for mandibular and parotid saliva, respectively, however, both flow rates appeared to be virtually stable after 2 h of stimulated salivation. Protein levels in the samples declined substantially in the first 60 min but remained at almost constant levels thereafter. The parotid salivary protein level (4·97 ± 4·41 mg mL^{-1}, n = 73) was always higher than the mandibular level (1·28 ± 1·15 mg mL^{-1}, n = 61). Salivary pH values for both saliva samples, however, did not change with time; the mean salivary pH was significantly higher in mandibular (7·89 ± 0·12, n = 30) than in parotid saliva (7·33 ± 0·08, n = 16).

Plasma and salivary concentrations of procainamide and NAPA after intravenous injection of procainamide

Fig. 1 shows the plasma and salivary concentration-time curves for the drug and NAPA. Each procainamide level declined bi-exponentially with time in almost parallel fashion. There was no significant difference between the level of drug in parotid saliva and in plasma. NAPA levels in parotid saliva tended to be higher than in mandibular saliva, and both levels appeared to decline in parallel with the plasma level throughout the experiment. Least-squares regression analysis of procainamide concentration (C) in each sample according to the equation, $C = Ae^{-\alpha t} + Be^{-\beta t}$, where A, B, α and β are hybrid parameters, yielded the estimates for these parameters as listed in Fig. 1; there were no differences in α and β values for plasma and saliva samples.

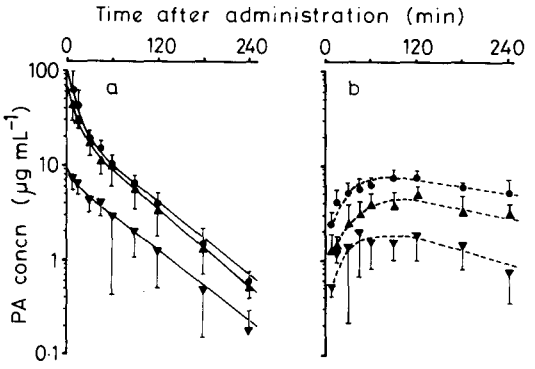

FIG. 1. (a) Plasma (●) and salivary (parotid (Pr), ▲; mandibular (M), ▼) procainamide (PA) concentrations after intravenous injection of PA hydrochloride (50 mg kg^{-1} as base) to rats that were stimulated for salivation with constant rate pilocarpine infusion (9·0 mg h^{-1} kg^{-1}). Each point is the mean ± s.d. of 3 to 5 rats. The solid line represents the computer-fitted bi-exponential curves weighted with unity. Estimated pharmacokinetic parameters are as follows. In plasma, A = 89·1 ± 6·1 μg mL^{-1}; B = 23·3 ± 5·5 μg mL^{-1}; α = 0·0914 ± 0·0134 min^{-1}; β = 0·0143 ± 0·0031 min^{-1}. In Pr saliva, A = 49·4 ± 2·5 μg mL^{-1}; B = 22·9 ± 2·4 μg mL^{-1}; α = 0·0937 ± 0·0101 min^{-1}; β = 0·0157 ± 0·0014 min^{-1}. In M saliva, A = 24·4 ± 14·0 μg mL^{-1}; B = 6·95 ± 3·39 μg mL^{-1}; α = 0·0885 ± 0·0111 min^{-1}; β = 0·0141 ± 0·0024 min^{-1}. (b) Plasma and salivary *N*-acetylprocainamide concentrations; rats treated as in (a). Symbols as in (a). Broken line indicates the eye-fitted curve.

Correlation between plasma and salivary concentrations for procainamide or NAPA

There were relatively good correlations ($P < 0·01$) between plasma (X) and salivary (Y) concentrations for both drug and NAPA (Fig. 2). The regression line for the drug was Y = 0·108X + 1·96 and Y = 0·917X and for NAPA Y = 0·609X and Y = 0·228X in parotid and mandibular saliva, respectively. The slope for the drug in parotid saliva vs the plasma level was the steepest and fairly close to unity.

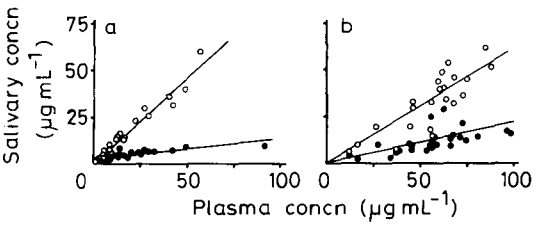

FIG. 2. Correlation between plasma (X) and salivary (Y) (parotid (Pr), ○; mandibular (M), ●) procainamide (PA, a) and *N*-acetylprocainamide (NAPA, b) concentration in rats. Regression lines are as follows: PA in Pr saliva; Y = 0·917X where r = 0·974 and n = 23; in M saliva; Y = 0·108X + 1·96 where r = 0·795 and n = 27. For NAPA, in Pr saliva; Y = 0·609X where r = 0·830 and n = 22; in M saliva; Y = 0·228X where r = 0·565 and n = 28. All correlations were significant at $P < 0·01$.

Plasma procainamide concentration which was predicted by the above regression line equation with the observed parotid salivary level at the corresponding time point, yielded almost the same estimates for the pharmacokinetic parameters (A = 53·8 ± 2·7 μg mL^{-1}, B = 24·9 ± 2·6 μg mL^{-1}, α = 0·0935 ± 0·0101 min^{-1}, β = 0·0157 ± 0·0014 min^{-1}) as did the original estimates from the plasma data itself (Fig. 1).

Prediction of S/P ratio by Matin's equation

In as much as procainamide and NAPA are weakly basic, the following equation (Matin et al 1974)

$$\frac{C_s}{C_p} = \frac{1 + 10^{(pKa - pH_s)}}{1 + 10^{(pKa - pH_p)}} \cdot \frac{f_p}{f_s}$$

may predict the saliva to plasma concentration ratio (S/P ratio), where pH_s and pH_p are pH values of

saliva and plasma, respectively; f_s and f_p are free fractions of total drug concentration in saliva and plasma, respectively. Table 1 summarizes the observed and predicted S/P ratios for drug and NAPA in both saliva samples according to the above equation, where pH_p = 7·40, pH_s = 7·33 (for parotid) or 7·89 (for mandibular), f_p = 0·688 (for drug and NAPA), f_s = 1·0 (for drug and NAPA) and pKa = 9·4 (Weily & Genton 1972). The plasma free fraction for procainamide was initially determined by equilibrium dialysis at two different concentrations (8 and 20 μg mL^{-1}), where the precise f_p value for NAPA could not be determined due to its slight instability during equilibrium dialysis for 12 h. For the prediction of the S/P ratio for NAPA, the same f_p value as that for procainamide was applied to the above equation. The salivary free fraction was found to be approximately equal to unity since the salivary protein level 2 to 6 h after constant rate infusion with pilocarpine (approximately 0·6 and 2·0 mg mL^{-1} in mandibular and parotid saliva, respectively) was almost negligible compared with the plasma protein level (approximately 70 to 80 mg mL^{-1}). Each predicted S/P ratio was considered to be fairly close to the corresponding observed value, since each prediction was almost within one s.d. from the mean observed value.

Table 1. Comparison of observed S/P ratio with calculated S/P ratio for both procainamide (PA) and *N*-acetylprocainamide (NAPA) in rats.

	S/P ratio			
	PA		NAPA	
	Pr[a]	M[b]	Pr	M
Observed	0·974 ± 0·243[c] (23)[d]	0·284 ± 0·119 (27)	0·589 ± 0·177 (21)	0·237 ± 0·117 (28)
Calculated[e]	0·807	0·227	0·795	0·274

[a] Parotid saliva.
[b] Mandibular saliva.
[c] Mean ± s.d.
[d] Number of data points.
[e] By Matin's equation.

DISCUSSION

The evidence for higher flow and lower protein level in mandibular saliva than in parotid saliva was consistent with that found in dogs (Watanabe et al 1981a) and rabbits (Watanabe et al 1985c). Furthermore, the initial protein level in each saliva was almost comparable with the respective mean measured after i.p. dosing with pilocarpine (8·0 mg kg^{-1}) in rats (Abe et al 1978). Although pH for the mixed saliva in rats has been reported to be approximately 7·8 to 7·9 (Ericsson 1962), individual pH values from either saliva sources have not been measured. The significant difference in pH (i.e. between pH_M and pH_{Pr}) of rat saliva was consistent with that found in the rabbit saliva (Watanabe et al 1985c). The present stimulation conditions employed in rats under constant rate pilocarpine (9·0 mg h^{-1} kg^{-1}) infusion were considered to be appropriate for the pharmacokinetic experiments of salivary drug excretion, since the flow, protein level and pH in both saliva sources were almost reproducible and stable from 2 to 6 h after infusion.

The parallel bi-exponential decline of each salivary procainamide level with the plasma level gave almost the same values for both α and β as those in the plasma sample, suggesting that the salivary drug level may be substituted for the plasma level if consistent S/P ratios are obtained over a broad concentration range. Since the procainamide level in parotid saliva was directly proportional to that in plasma, the plasma pharmacokinetics could be predicted almost completely. However, mandibular saliva, which had a significant Y-intersect in the correlation of the salivary and plasma drug concentrations (Fig. 2), failed to predict the plasma procainamide levels. The almost parallel time-course of each salivary NAPA level with the plasma level yielded a fairly good correlation without any Y-intersect. However, inability to analyse both plasma and salivary NAPA levels pharmacokinetically did not allow any predictions to be made.

In both mandibular and parotid saliva, Matin's equation could predict fairly close values for the mean S/P ratio of drug and NAPA, therefore, the two approximations that f_s in each saliva is equal to unity for both drug and NAPA and f_p for NAPA is the same as that for procainamide, were thought to be acceptable. Gland type difference (parotid or mandibular) in the S/P ratio for either drug or NAPA may then be attributed to difference in the salivary pH. Koup et al (1975) have reported the mean (±s.d.) S/P ratio of procainamide to be 3·50 (±2.34) in combined saliva from chronically medicated arrhythmic patients. Matin's equation using the reported salivary pH (6·8, Mandel 1974) and f_p (0·84, Reidenberg et al 1975) could also calculate the S/P ratio as 3·32, which was comparable with the reported value given above (Koup et al 1975).

In conclusion, pharmacokinetic investigation of the salivary excretion in rats of drugs such as procainamide or NAPA was found to be possible under the present experimental conditions, and it is

suggested that the S/P ratio for both drug and NAPA is dependent on the salivary pH.

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