J. Pharm. Pharmacol. 1993, 45: 154–156
Communicated April 7, 1992

Dependency of salivary excretion of mexiletine on the plasma concentration in rats

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Abstract—The effect of steady-state plasma concentrations on the salivary excretion of mexiletine was investigated following simultaneous bolus intravenous injection of the loading dose (2·7 or 16·1 mg kg^{-1}) and constant-rate intravenous infusion of the maintenance dose (15 or 102 $\mu g\ min^{-1}\ kg^{-1}$) in male Wistar rats. Parotid and mandibular saliva was collected separately by stimulating salivation with a constant-rate infusion of pilocarpine (50 $\mu g\ kg^{-1}\ min^{-1}$) in each rat. The low and high steady-state levels of mexiletine in blood plasma were attained at $0{\cdot}259 \pm 0{\cdot}123$ and $1{\cdot}616 \pm 0{\cdot}475\ \mu g\ mL^{-1}$, respectively, within the first 1–2 h after drug administration. Similarly, the two different steady-states in both parotid and mandibular saliva were attained. Although the mexiletine levels in both types of saliva were lower than that in plasma, the drug level in parotid saliva was always higher than that in mandibular saliva at any steady-state ($P < 0{\cdot}001$ or $0{\cdot}01$). In parotid saliva, the high steady-state produced greater saliva to plasma drug concentration ratios (S/P ratio, $0{\cdot}475 \pm 0{\cdot}160$) than that ($0{\cdot}386 \pm 0{\cdot}131$) at the low steady-state ($P < 0{\cdot}05$). The S/P ratio for mandibular saliva at the high ($0{\cdot}204 \pm 0{\cdot}060$) steady-state was also greater than that at the low ($0{\cdot}158 \pm 0{\cdot}050$) steady-state ($P < 0{\cdot}01$). These changes in the S/P ratio could not be explained by the pH for either parotid or mandibular saliva, but partially by the change in the unbound fraction of the drug which tended to be consistent with that in the ratio for both salivary glands. These findings suggest that the salivary excretion of mexiletine may be dependent on the plasma unbound concentration in rats.

It has been reported that mexiletine was excreted into saliva with higher concentrations than those in plasma or serum of man (Beckett & Chidomere 1977; Katagiri et al 1989, 1991). However, no detailed pharmacokinetic study has been carried out to evaluate the salivary excretion mechanism of mexiletine except for our recent investigations in rats. We have reported (Nagasako et al 1992a, b) that higher plasma drug concentrations in the distribution phase tended to produce relatively large saliva to plasma concentration ratios (S/P ratios) for both parotid and mandibular saliva following bolus intravenous administration of mexiletine at 5, 10 or 15 mg kg^{-1}, that the S/P ratio for parotid saliva tended to be consistently greater after the dose of 15 mg kg^{-1} than after 5 mg kg^{-1}, and that similar dose-dependency was observed in mandibular saliva during the initial distribution phase. These results suggested a possible concentration-(or dose)-dependency in the salivary excretion of mexiletine.

The present study was to evaluate a dependency of salivary excretion of mexiletine on the plasma concentration in rats.

Materials and methods

Materials. Mexiletine hydrochloride was kindly supplied by Boehringer Ingelheim Japan Co. Ltd (Kawanishi, Japan). Fluorescamine used for fluorometric derivatization was purchased from F. Hoffman-La Roche Co. Ltd (Basle, Switzerland). 1-Pentane sulphonic acid (Pic B-5) employed as an ion-pairing reagent was obtained from Waters (Milford, USA). All other reagents and solvents were of analytical grade. A constant-rate infusion pump (KN-201, Natsume Seisakusho Co. Ltd, Tokyo, Japan) was used for the infusion of pilocarpine and mexiletine. A compact type of pH meter with combined electrode (C-1, Horiba Seisakusho Ltd, Kyoto, Japan) was used for an immediate measurement of saliva pH in small samples (80–100 μL).

Animals. Male Wistar rats (340–410 g, 12–13 weeks old) were anaesthetized with pentobarbitone (50 mg kg^{-1}, i.p.) after overnight fasting for 12 h. Body temperature was kept at 37·5°C by using a heated pad placed under the supine rat.

Drug administration and collection of blood and saliva samples. After tracheotomy and catheterization, cannulae were made according to the method of Watanabe et al (1987). The femoral vein was cannulated with a polyethylene tubing (PE-50) for infusion of pilocarpine hydrochloride at a constant-rate of 50 μg (free base) $kg^{-1}\ min^{-1}$ to stimulate salivation and for constant-rate infusion of a maintenance dose of mexiletine. The jugular vein was also cannulated with silicone polymer tubing (i.d. 1·0 mm; o.d. 1·5 mm, Dow Corning, Tokyo, Japan) for bolus administration of mexiletine and for collection of blood samples. Bevelled polyethylene tubing (PE-10) was inserted into the parotid and mandibular duct orifices in the buccal cavity to collect saliva samples separately. Following constant-rate infusion of pilocarpine for 2 h to stabilize the salivation (Watanabe et al 1987), mexiletine was administered intravenously as a bolus loading dose (D_0) followed by a constant-rate infusion of a maintenance dose (k_0) over 6 h. In order to attain immediately the desired steady-state drug concentration in plasma (C_{ss}), D_0 was chosen according to the recommendation of Mitenko & Ogilvie (1972) as follows

$$D_0 = k_0/\beta$$

where $k_0 = C_{ss} \cdot CL_{tot}$, assuming linear pharmacokinetics for plasma mexiletine. For this purpose, we employed the parameters which were estimated for plasma mexiletine following the bolus intravenous administration (5 and 15 mg kg^{-1}) to rats in our previous report indicating linear pharmacokinetics (Nagasako et al 1992b). The doses at two levels with the desired C_{ss} and values used are listed in Table 1. Saliva samples were collected periodically for 20 min. Blood samples (0·13 mL) were withdrawn at the midpoint of the periodical saliva collection intervals and were centrifuged to obtain plasma after heparinize-

Table 1. Calculated doses of mexiletine using pharmacokinetic parameters obtained following bolus intravenous administration of mexiletine to rats.

	Dose (mg kg^{-1})	
Parameters	5	15
C_{ss} ($\mu g\ mL^{-1}$)	0·200	1·50
β[a] (min^{-1})	0·00557	0·00633
CL_{tot}[a] ($mL\ min^{-1}\ kg^{-1}$)	74·3	67·9
k_0 ($\mu g\ min^{-1}\ kg^{-1}$)	14·9	101·9
D_0 (mg kg^{-1})	2·68	16·1
Total dose (mg kg^{-1})	8·2	52·8

[a] These values were obtained following bolus intravenous administration of mexiletine to rats (Nagasako et al 1992b).

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tion. For the measurement of saliva pH, saliva samples were collected under a liquid paraffin layer (about 0·1 mL) between 5 and 6 h from the same rats as used for the above kinetic experiments. Salivary flow rate was determined gravimetrically assuming the specific gravity of saliva to be approximately 1·0 (Watanabe et al 1981). In the serum protein binding experiments performed by the same method as the steady-state experiments described above, about 5 mL of blood was withdrawn from the rats (n = 4–5), 2 h after drug administration. The binding of mexiletine to serum protein was determined as described previously (Nagasako et al 1992b).

Analytical procedures. Concentrations of mexiletine in plasma and saliva were determined by HPLC (Nagasako et al 1992a).

Data analysis. The statistical significance of the data was evaluated by using Student's *t*-test.

Results

Plasma and saliva mexiletine concentrations on simultaneous bolus intravenous injection of a loading dose and constant-rate intravenous infusion of a maintenance dose. The concentration-time profiles of mexiletine for plasma, parotid and mandibular saliva are shown in Fig. 1. The low and high steady-state levels of mexiletine in plasma were attained at 0·259 ± 0·123 and 1·616 ± 0·475 μg mL^{-1}, respectively, within the first 1–2 h. The steady-state levels of mexiletine in both parotid and mandibular saliva were also attained within the first 1–2 h. Although the drug levels in both types of saliva were lower than that in plasma, the steady-state levels in parotid saliva (low, 0·089 ± 0·021 μg mL^{-1}; high, 0·737 ± 0·257 μg mL^{-1}) were always higher ($P < 0·01$) than those in mandibular saliva (low, 0·037 ± 0·009 μg mL^{-1}; high, 0·310 ± 0·063 μg mL^{-1}).

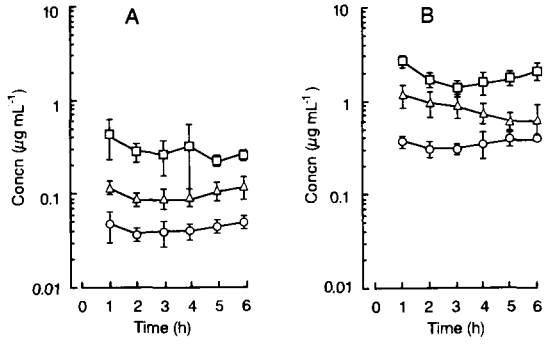

FIG. 1. Plasma and saliva concentrations of mexiletine during constant-rate infusion of mexiletine at low (5 mg kg^{-1} A) or high (15 mg kg^{-1} B) dose in rats. Each point and vertical bar represent the mean and s.d. of six rats. □, Plasma; △, parotid saliva; ○, mandibular saliva.

Mexiletine binding to serum protein at low and high steady-states was 62·3 ± 5·6 and 54·6 ± 5·2%, respectively. This difference was not significant but indicated only a trend for an increase of unbound drug at the higher drug levels (Table 2).

Saliva pH and flow rate. Saliva pH and flow rates of parotid and mandibular saliva are summarized in Table 2. The high steady-state produced the greater value of pH for both parotid and mandibular saliva. In addition, there was a consistent tendency for the pH of parotid saliva to be lower than that of mandibular saliva at any steady-state. The flow rates for both parotid and mandibular saliva were significantly reduced at the high steady-state ($P < 0·01$). Furthermore, the flow rate for mandibular saliva was significantly larger than that for parotid saliva at any steady-state ($P < 0·01$).

Effect of plasma concentration of mexiletine on S/P ratio. The mean values of S/P ratio for each saliva are shown in Table 3. The ratios in both parotid and mandibular saliva at high steady-state were significantly greater than those at the low steady-state ($P < 0·05$ or 0·01). In addition, the S/P ratios in parotid saliva were significantly greater than those in mandibular saliva at any steady-state ($P < 0·001$ or 0·01).

Table 3. S/P ratio of mexiletine during constant-rate intravenous infusion in rats.

	Dose (mg kg^{-1})	
Saliva	5	15
Parotid	0·386 ± 0·131 (36)	0·475 ± 0·160[a] (36)
Mandibular	0·158 ± 0·050[b] (36)	0·204 ± 0·060[cd] (36)

Each value represents the mean ± s.d. Number of data points is shown in parentheses. [a]Significantly different from the low dose at $P < 0·05$. [b]Significantly different from parotid saliva at $P < 0·001$. [c]Significantly different from the low dose at $P < 0·01$. [d]Significantly different from parotid saliva at $P < 0·01$.

Table 2. Saliva pH, salivary flow rate, salivary clearance and unbound fraction in serum protein.

		Dose (mg kg^{-1})	
	Saliva	5	15
Saliva pH	Parotid	8·02 ± 0·10 (4)	8·37 ± 0·02[a] (4)
	Mandibular	8·17 ± 0·10 (5)	8·48 ± 0·06[a] (6)
Saliva flow rate (μL min^{-1} kg^{-1})	Parotid	23·08 ± 12·68 (36)	10·46 ± 7·16[b] (36)
	Mandibular	37·21 ± 25·85[c] (36)	19·76 ± 7·88[bc] (36)
Salivary clearance[d] (μL min^{-1} kg^{-1})	Parotid	8·36 ± 5·33 (36)	5·42 ± 4·65 (36)
	Mandibular	5·90 ± 4·55 (36)	4·29 ± 2·66 (36)
Unbound fraction		0·377 ± 0·050 (5)	0·454 ± 0·052 (4)

Each value represents the mean ± s.d. Number of rats or data points is shown in parentheses. [a] Significantly different from the low dose at $P < 0·01$. [b] Significantly different from the low dose at $P < 0·01$. [c] Significantly different from parotid saliva at $P < 0·01$. [d] Calculated by multiplying the S/P ratio by the flow rate.

Discussion

Investigations on the excretion mechanism of drugs into saliva have been limited to only a few drugs (Watanabe et al 1987; Iwamoto et al 1988; Hayashi et al 1988, 1989; Hayashi & Watanabe 1990). Only one report has been published for the salivary excretion mechanism of a weak basic drug, procainamide (Iwamoto et al 1988). From the results in our previous reports with mexiletine (Katagiri et al 1989, 1991; Nagasako et al 1992a, b), a weak base, we determined a need to examine the salivary excretion profiles of this drug at different steady-state plasma concentrations. Mitenko & Ogilvie (1972) reported that by selecting bolus injection and simultaneous, continuous infusion doses based on the appropriate pharmacokinetic parameters, constant plasma concentrations could be obtained within the first hour. In the present study, employing this method, low and high steady-state concentrations of plasma mexiletine were attained within the first 1–2 h (Fig. 1). The small discrepancy of the achieved steady-state from the expected value (Table 1) may be due to variations in the estimates for pharmacokinetic parameters (Nagasako et al 1992b). The drug concentrations in both types of saliva also attained their steady-state levels within 1–2 h after each dose (Fig. 1).

In the present study, the S/P ratios of parotid saliva were significantly larger than those of mandibular saliva, whereas the pH values in parotid saliva tended to be lower than those in mandibular saliva at any steady-state. According to Matin et al (1974), a decrease of saliva pH results in a greater S/P ratio for a weak base. Thus, a glandular difference in the S/P ratio of mexiletine was qualitatively explained by this difference in saliva pH.

Hayashi et al (1989) reported that the S/P ratios of 5-fluorouracil for both parotid and mandibular saliva were larger at a high steady-state concentration than at a low steady-state concentration, suggesting that the plasma concentration or dose of this drug may influence the S/P ratio, in some saturation kinetic manner, during constant rate intravenous infusion in rats. In the present study with mexiletine, the S/P ratios for both parotid and mandibular saliva at the high steady-state were significantly greater than those at the low steady-state ($P < 0.05$ and 0.01, Table 3). These findings confirmed our previous results (Nagasako et al 1992a, b) that the higher plasma concentration of mexiletine after the bolus dose yielded the larger S/P ratio. These changes in the S/P ratios at two different steady-states could not be explained by the change in pH for any salivary gland (Table 2). In contrast, it was found that the change in the fraction of unbound drug to serum protein at the two steady-states gave the same trend as the change in the S/P ratios for both parotid and mandibular saliva. These aspects suggest that the plasma unbound concentration of mexiletine may influence the salivary excretion of this drug in both glands.

The salivary clearance is a function of the S/P ratio and flow rate. In spite of the greater flow rate in mandibular saliva at both low and high steady-states (Table 2), the salivary clearance for mandibular gland tended to be smaller than that for parotid gland. This result was considered to be directly caused by the gland-type difference in the S/P ratio. In addition, the high steady-state tended to reduce the salivary clearance despite an increase in the S/P ratio. This was due to the significant decrease in the flow rate for both parotid and mandibular saliva (Table 2). The reduction in the salivary flow was probably caused by one of the pharmacological effects of mexiletine, since the drug has been reported to give rise to a slight reduction in carbachol-induced salivation in mice (Kitagawa et al 1983).

From the present study, it is suggested that the salivary excretion of mexiletine may be dependent on the plasma unbound concentration. The present findings supported the previous observations after the bolus doses (Nagasako et al 1992a, b).

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