International Journal of Pharmaceutics, 81 (1992) 127–132

IJP 02687

Effect of dose on salivary excretion of mexiletine after bolus intravenous administration in rats

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(Received 29 June 1991)
(Modified version received 2 September 1991)
(Accepted 22 October 1991)

Key words: Mexiletine; Saliva; Dose dependence; Plasma concentration dependence; Saliva/plasma concentration ratio; Salivary clearance; Rat

Summary

The effect of dose on the salivary excretion of mexiletine was investigated after bolus intravenous administration of the drug (5 and 15 mg/kg) in rats. Parotid (Pr) and mandibular (M) saliva was collected separately by stimulating salivation through the infusion of pilocarpine at a constant rate (3 mg/kg per h). The mexiletine levels in blood plasma, Pr and M saliva declined bi-exponentially with time in almost parallel fashion. The drug levels in both types of saliva were lower than that in plasma, the concentration in Pr saliva always being greater than that in M saliva. Although the pharmacokinetic parameters for α, β or $t_{1/2\beta}$ in plasma and each form of saliva were almost identical for the two dose levels, the saliva to plasma concentration (S/P) ratios were compared between Pr and M saliva in the distribution phase vs the elimination phase after the administration of both doses. A consistent trend was observed where all S/P ratios were relatively high after the dose of 15 mg/kg except that for M saliva in the elimination phase. Furthermore, at both dose levels, plasma drug concentrations in the distribution phase resulted in significantly greater S/P ratios in both Pr ($P < 0.001$) and M ($P < 0.05$) saliva as compared to those in the elimination phase. These findings suggest the possibility that the extent of salivary excretion of mexiletine may depend upon the dose (or concentration) despite the lack of a dose effect on the pharmacokinetic parameters in both plasma and saliva.

Introduction

Among extensive studies on the salivary excretion of drugs, several reports have recently appeared in which the mechanisms of salivary excretion of weak acidic drugs were investigated in detail using experimental animals (Watanabe et al., 1985; Hayashi et al., 1988a,b, 1989; Hayashi and Watanabe, 1990). Hayashi et al. (1988b) reported that the saliva to plasma concentration (S/P) ratio of 5-fluorouracil in parotid (Pr) and mandibular (M) saliva was greater at higher doses and decreased with the decline in plasma concentrations following the bolus intravenous administration of 5-fluorouracil in rats. However, no studies on the mechanism of salivary excretion of weak basic drugs have been published except those on procainamide (Watanabe et al., 1987; Iwamoto et al., 1988).

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In previous papers, we reported that the therapeutic monitoring of serum concentrations of the anti-arrhythmic drug, mexiletine, which is weakly basic, may be substituted by following the saliva levels, but that the salivary excretion of this drug could not be quantitatively explained by simple, passive secretion mechanisms based on pH-partition theory in either humans or rats (Katagiri et al., 1989, 1991; Nagasako et al., 1992). This drug tended to yield relatively high S/P ratios for both Pr and M saliva in rats at higher plasma levels of drug in the distribution phase (Nagasako et al., 1992). The earlier results suggested the possibility that the salivary excretion of mexiletine may be dependent on dose (or concentration).

In the present study, the effects of the dose (or plasma levels) of mexiletine on the kinetics of its salivary excretion and the S/P ratios were investigated following bolus intravenous administration at two dose levels, 5 and 15 mg/kg, in rats from which saliva from two different sources, i.e., Pr and M saliva, was collected separately.

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-Pentanesulfonic acid (Pic B-5) employed as an ion-pairing reagent was obtained from Waters (Milford, U.S.A.). All other reagents and solvents were commercial products of analytical grade.

Animals

Male Wistar rats (weighing 360–380 g, 12 weeks old) were anesthetized with pentobarbital (50 mg/kg i.p.) after overnight fasting for 12 h. Body temperature was maintained thermostatically at 37.5°C by using a heated pad placed under the supine rats.

Drug administration and collection of blood and saliva samples

After tracheotomy and catherization, cannulae were made according to the method described by Watanabe et al. (1987). The femoral vein was cannulated with polyethylene tubing (PE-50) for infusion of pilocarpine hydrochloride at a constant rate of 3.0 mg (free base)/kg per h to stimulate salivation during the experiment. 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 administration of mexiletine and for collection of blood samples. Beveled polyethylene tubing (PE-10) was inserted into the mandibular (M) and parotid (Pr) duct orifices in the buccal cavity to collect saliva samples separately. Following the infusion of pilocarpine at a constant rate for the initial 2–3 h (Watanabe et al., 1987), mexiletine was administered intravenously as a bolus dose of 5 or 15 mg/kg to rats ($n = 5$ or 6). Samples of saliva were removed at intervals over a period of 20 min. Blood samples (0.13 ml) were withdrawn midway through the period of saliva collection and then centrifuged to obtain plasma after heparinization. For serum protein binding experiments, about 5 ml of blood was withdrawn from the other rats ($n = 6$) at 10 min and 4 h after i.v. administration. Serum was immediately separated from blood by centrifugation using a serum separator (Fibrichin, Takazono Sangyo Co. Ltd, Osaka, Japan), and an aliquot (0.8 ml) of serum was employed for ultrafiltration by using a micropartition system MPS-3 (Amicon Corp., Danvers, MA, U.S.A.). The binding of mexiletine to serum protein was determined by assaying drug concentrations in the serum and its filtrate.

Analytical procedures

The concentrations of mexiletine in plasma and saliva samples were determined by a high-performance liquid chromatography (HPLC) method as described previously (Nagasako et al., 1991).

Data analysis

Analysis of the plasma and saliva mexiletine concentration-time data was performed according

to the two-compartment model with rapid intravenous administration by the nonlinear least-squares computer program MULTI (Yamaoka et al., 1981). Statistical evaluation of data was carried out using Student's t-test.

Results

Plasma and saliva concentrations of mexiletine after bolus intravenous administration

Fig. 1 shows the mexiletine concentration-time profiles for plasma, and both Pr and M saliva after bolus i.v. administration of the drug at 5 and 15 mg/kg into rats. After both doses, the drug concentrations in these biological fluids decreased bi-exponentially with time in almost parallel fashion. Although the mexiletine levels in both types of saliva were consistently below those in plasma, Pr saliva showed higher drug levels than M saliva at all time points. The pharmacokinetic parameters in plasma and each form of saliva estimated by using the MULTI program, are summarized in Table 1. The values for α, β and $t_{1/2\beta}$ in plasma were almost equal for both dose levels. Furthermore, estimates of α, β or $t_{1/2\beta}$ in both Pr and M saliva also yielded an identical value for the two doses, each estimate also being scarcely different from the corresponding value determined in plasma.

The fractions of mexiletine bound at 10 min (initial distribution phase) and 4 h (terminal elimination phase) after administration of a dose of 5 mg/kg were 66.2 ± 5.0 and $64.4 \pm 4.6\%$, respectively, and 54.3 ± 4.9 and $56.3 \pm 4.3\%$, respectively, for the dose of 15 mg/kg. Although the fraction of mexiletine bound was significantly different between the two doses at both time points (10 min, $P < 0.01$; 4 h, $P < 0.05$), no significant difference was found in the fraction of drug bound at the two time points after each dose.

Effect of dose and plasma concentration of mexiletine on S/P ratio

The S/P ratios for Pr and M saliva in the initial distribution (0–50 min) and terminal elimination (2–6 h) phases after the two doses are compared in Table 2. Although there was no significant difference in the S/P ratio for both Pr and M saliva at the two dose levels, all S/P ratios tended to be elevated following the dose of 15 mg/kg except that of M saliva in the elimination phase. Regarding the effect of plasma concentration on the S/P ratio at each dose level,

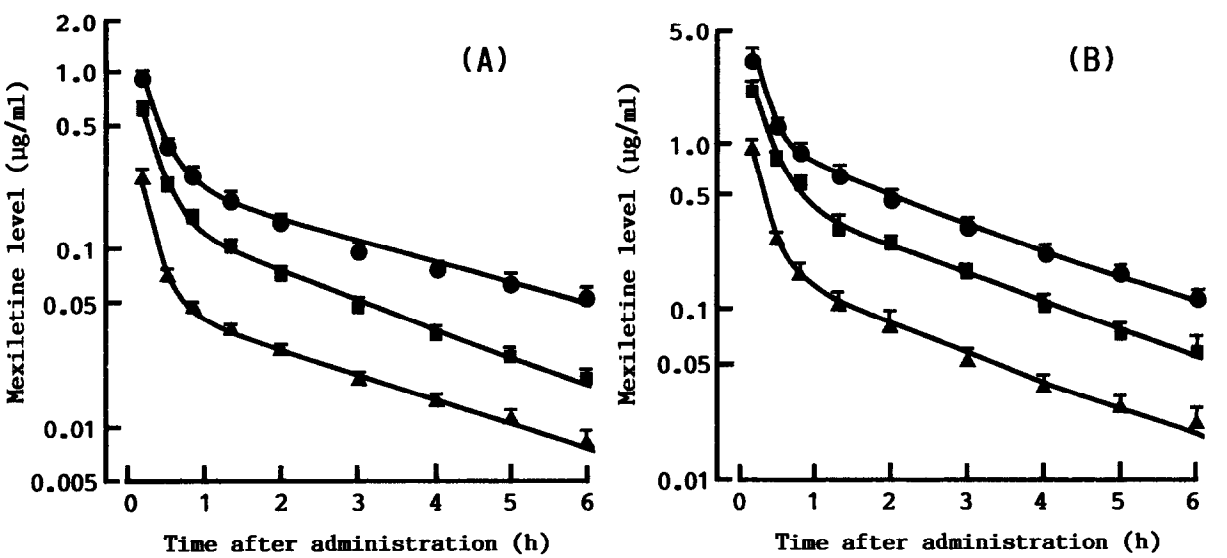

Fig. 1. Plasma and saliva levels of mexiletine after bolus intravenous administration of 5 (A) and 15 (B) mg/kg of the drug in rats. Each point and vertical bar represent the mean and S.D. of 4–6 rats. (●) Plasma; (■) Pr saliva; (▲) M saliva.

TABLE 1

Pharmacokinetic parameters for plasma and saliva mexiletine after bolus intravenous administration at two dose levels in rats

Parameter [a]	5 mg/kg			15 mg/kg		
	Plasma	Pr saliva	M saliva	Plasma	Pr saliva	M saliva
A (μg/ml)	1.42 ± 0.33	–	–	5.87 ± 2.15	–	–
B (μg/ml)	0.279 ± 0.051	–	–	1.06 ± 0.35	–	–
α ($\times 10^{-2}$) (min^{-1})	7.66 ± 1.78	8.29 ± 2.53	10.03 ± 0.92	8.48 ± 1.31	8.44 ± 0.85	9.10 ± 1.98
β ($\times 10^{-3}$) (min^{-1})	5.57 ± 1.47	6.98 ± 1.28	5.64 ± 0.63	6.33 ± 0.48	7.79 ± 0.75	6.63 ± 0.86
$t_{1/2\beta}$ (min)	129.4 ± 28.4	101.9 ± 16.9	124.2 ± 14.0	110.0 ± 8.80	89.6 ± 8.5	105.9 ± 12.9

Each value represents the mean ± S.D. of 5 or 6 rats.
[a] Estimated by the MULTI program [weight(i) = $1/Ci$].

TABLE 2

Comparison of the S/P ratios for Pr and M saliva between the initial distribution phase and terminal elimination phase at two dose levels

Saliva	Dose (mg/kg)	S/P ratio	
		Distribution phase	Elimination phase
Pr	5	0.614 ± 0.125	0.458 ± 0.096 [a]
	15	0.662 ± 0.114	0.500 ± 0.123 [a]
M	5	0.219 ± 0.061	0.186 ± 0.032 [b]
	15	0.243 ± 0.098	0.171 ± 0.058 [b]

Each value represents the mean ± S.D. of 5 or 6 rats.
[a] Significantly different from the value in the distribution phase at $P < 0.001$.
[b] Significantly different from the value in the distribution phase at $P < 0.05$.

TABLE 3

Comparison of the salivary clearance for Pr and M saliva between the initial distribution phase and terminal elimination phase at two dose levels

Saliva	Dose (mg/kg)	Salivary clearance (μl/min per kg)	
		Distribution phase	Elimination phase
Pr	5	23.33 ± 6.36	17.50 ± 3.75 [a]
	15	17.95 ± 8.54 [b]	14.25 ± 6.54 [b]
M	5	8.21 ± 5.13	12.41 ± 4.39 [a]
	15	7.73 ± 4.91	9.47 ± 6.21 [b]

Each value represents the mean ± S.D. of 5 or 6 rats. All values in Pr saliva were significantly greater than the corresponding values in M saliva.
[a] Significantly different from the value in the distribution phase at $P < 0.01$.
[b] Significantly different from the value at the dose of 5 mg/kg at $P < 0.05$.

the ratios for Pr and M saliva determined in the initial distribution phase were significantly greater as compared to those in the terminal elimination phase (Pr, $P < 0.001$; M, $P < 0.05$).

Effect of dose and plasma concentration of mexiletine on salivary clearance

The estimates for Pr and M salivary clearances in the initial distribution and terminal elimination phases are listed in Table 3. Salivary clearance has previously been defined as the rate of salivary drug excretion divided by the plasma concentration of drug (Watanabe et al., 1984) and was calculated for each set of periodical data. Although the values for the clearance in both types of saliva tended to undergo considerable fluctuation at both dose levels, all values in Pr saliva were significantly greater than those in M saliva. Both Pr and M salivary clearances were significantly enhanced or tended to become greater after the dose of 5 mg/kg. In Pr saliva, clearance tended to increase in the distribution phase after both doses, whereas the opposite trend was observed for M saliva.

Discussion

In attempting to utilize saliva drug levels for routine therapeutic monitoring by substituting for serum (or plasma) drug levels, various pilot studies have been carried out during the preceding two decades. In addition, several reports have recently been published on the mechanisms of excretion of acidic drugs into saliva using experi-

mental animals (Watanabe et al., 1981a,b, 1985; Hayashi et al., 1988a,b, 1989; Hayashi and Watanabe, 1990). However, investigations on the mechanism of salivary excretion of the weakly basic drug, procainamide are scarce in the literature (Watanabe et al., 1987; Iwamoto et al., 1988). In the present study, the levels of mexiletine in Pr and M saliva were lower than those in plasma following both doses of 5 and 15 mg/kg. This finding is consistent with our data reported in a previous paper (Nagasako et al., 1992). The drug level in Pr and M saliva declined in parallel with the plasma level after both doses (Fig. 1), yielding almost identical values for estimates of α, β and $t_{1/2\beta}$ in saliva as well as those in plasma after such low and high doses (Table 1). Therefore, the results suggested that the dose level had no significant influence on the pharmacokinetic parameters in plasma and either Pr or M saliva.

It has been reported that the S/P ratio of 5-fluorouracil is affected by its plasma concentration as well as by the dose given to rats, namely, the higher the plasma level or dose, the greater the S/P ratio (Hayashi et al., 1988b, 1989). In the current study, with mexiletine at 5 and 15 mg/kg as a bolus i.v. dose, the S/P ratios for Pr and M saliva in the initial distribution phase were significantly higher than those in the terminal elimination phase (Pr, $P < 0.001$; M, $P < 0.05$, Table 2). A similar result was obtained in our previous report (Nagasako et al., 1992). Furthermore, we observed a consistent trend of all the S/P ratios being larger after the dose of 15 mg/kg as compared to 5 mg/kg, except in the case of M saliva in the elimination phase (Table 2). This result may be partly attributable to an increase in the non-bound fraction of mexiletine after the dose of 15 mg/kg as compared with that of 5 mg/kg. However, differences in the S/P ratio for Pr or M saliva between the initial distribution and terminal elimination phases could not be explained on the basis of such protein binding phenomena. The above findings suggest that the salivary excretion of mexiletine is dependent to a small extent on the dose or on the concentration of mexiletine in plasma rather than being a process of simple, passive secretion based on pH-partition theory. This aspect appears to be inconsistent with the apparent linearity determined for the pharmacokinetic parameters of plasma and both forms of saliva.

Hayashi et al., (1988b) reported that the salivary clearance of 5-fluorouracil for both Pr and M saliva tended to increase with higher dose, suggesting that the salivary excretion of 5-fluorouracil was influenced by the dose. In contrast, the present study with mexiletine has demonstrated the opposite trend for the salivary clearance of the drug for both Pr and M saliva, i.e., a decrease at the higher dose, 15 mg/kg (Table 3). Significant decreases in salivary flow rates (μl/min per kg) for both Pr and M saliva were found to occur with increase in the dose from 5 to 15 mg/kg (Pr saliva, 38.99 ± 8.74 to 30.22 ± 14.71, $P < 0.001$; M saliva, 54.39 ± 27.16 to 43.31 ± 27.01, $P < 0.05$). Mexiletine has been reported to give rise to a slight reduction in carbachol-induced salivation in mice (Kitagawa et al., 1983). Therefore, the effect of dose on the salivary clearance of mexiletine may be mediated by a decrease in salivary flow rate induced by a higher dose (15 mg/kg) of the drug, since clearance is a function of the flow rate.

From the present study, it is suggested that mexiletine has a weak tendency to be excreted into the saliva in a dose- or concentration-dependent manner. The present findings are considered to support previous observations in humans (Beckett and Chidomere, 1977; Katagiri et al., 1989, 1991).

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