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Salivary excretion of mexiletine in normal healthy volunteers

YOSHIHIRO KATAGIRI, SADAO NAGASAKO, MASAKAZU HAYASHIBARA, KIKUO IWAMOTO, *Department of Pharmacy, Shimane Medical University Hospital, 89-1 Enya-cho, Izumo 693, Japan*

Abstract—To investigate the kinetics and correlation between serum and saliva levels of mexiletine, serum (total and unbound) and saliva drug concentration-time courses have been analysed in five normal healthy volunteers after administration of a single oral dose (200 mg) of the drug. Mexiletine levels in saliva were always higher than those in serum. The drug concentration-time curve in each sample was analysed according to the non-linear least squares regression program MULTI, for a two-compartment model with first-order absorption. The saliva drug concentration in the post-absorption phase was found to be well correlated with either corresponding serum total or serum unbound drug level in four of the subjects. Although there was a large inter-individual variation in the ratio of saliva to serum drug concentrations as well as in the pharmacokinetic parameters, an almost consistent ratio was obtained in each individual.

Mexiletine is a type Ib anti-arrhythmic drug, frequently used to treat ventricular arrhythmia. Since the drug's therapeutic range is relatively narrow (0·5–2·0 μg mL^{-1}), monitoring of blood concentration is necessary. However, the long-term, repetitive collection of blood samples from severe arrhythmic patients is unacceptible.

Recently, attention has been paid to the use of saliva samples, in the place of blood samples, for drug monitoring (Graham 1986). Drug levels in saliva are usually well related to the pharmacological activity, since the level of a drug in saliva is usually equal to the protein-unbound levels in the serum or plasma (Danhof & Breimer 1978). However, the kinetics of salivary excretion and the correlation between the saliva and serum concentrations of mexiletine has not been extensively studied.

In this study, we have investigated the salivary excretion kinetics of mexiletine as well as the serum concentration-time courses after the oral administration of the drug in normal healthy volunteers. In addition, we have compared the drug levels in the saliva with those in the serum to find if it is possible to use saliva samples in the place of serum samples for therapeutic drug monitoring.

Materials and methods

Materials. Mexiletine hydrochloride was supplied by Boehringer Ingelheim Japan Co. Ltd (Kawanishi, Japan) and Mexitil capsules were used as the dosage form. Fluorescamine, for fluorometric derivatization, was purchased from F. Hoffman-La Roche Co. Ltd (Basle, Switzerland). 1-Pentane sulphonic acid (Pic B-5), an ion-pairing reagent, was purchased from Waters (Milford, USA). All other reagents used were of analytical grade.

Study protocol. The study was approved by the Local Division of the Institutional Review Board of Shimane Medical University Hospital and informed consent was given by all subjects.

Five normal male volunteers (32·4 ± 2·8 years old, 63·8 ± 5·1 kg) were given orally two Mexitil capsules (200 mg mexiletine) at 0800 h, with 200 mL of water. Blood and saliva samples were collected at 1, 2, 3, 4, 6, 8, 12 and 24 h after administration. Standardized meals for normnal healthy Japanese subjects were taken at 0740, 1300 and 1900 h. Blood samples (5 mL) were periodically withdrawn from the cephalic vein at the mid-point of the corresponding period for saliva collection. Serum was immediately obtained from the blood by centrifugation with a serum separator, Fibrichin (Takazono Sangyo Co. Ltd, Osaka, Japan). Mixed saliva (i.e. whole saliva) was collected for 5 min by means of continuous mouth and tongue movement (Katagiri et al 1989). Pre-saliva was discarded before the periodical saliva collection. Saliva pH and flow rate were determined as reported previously, and were unaffected by the administration of the drug compared with the corresponding control values (Katagiri et al 1989). Collected saliva samples were immediately centrifuged to remove the mucosal tissue debris.

Correspondence: K. Iwamoto, Department of Pharmacy, Shimane Medical University Hospital, 89-1, Enya-cho, Izumo 693, Japan.

Analytical method. Mexiletine in serum and saliva was determined using the HPLC method of Grech-Bélanger et al (1984) with modifications in the sample preparation by the extraction method. A sample (200 μL) of serum or saliva was treated as reported previously (Katagiri et al 1989); after fluorescent derivatization of mexiletine in the extracted residue with 50 μL of fluorescamine solution in acetone (0·25 mg mL^{-1}), a 10 μL sample was injected into the HPLC system. Serum unbound fraction was separated by ultracentrifugation with Centrisart I (Sartorius GmbH, Göttingen, Germany) and also used for HPLC analysis.

Kinetic analysis. The individual serum and saliva concentration-time data were analysed according to the two-compartment model with first-order absorption using the program MULTI (Yamaoka et al 1981). Correlation between serum and saliva drug concentrations in the post-absorption phase was examined in each subject. The saliva to serum (total) concentration ratio (saliva/serum ratio) for mexiletine was also estimated in each subject. Significant differences were quantified by Student's *t*-test.

Results

Serum and saliva mexiletine concentration-time courses. Mexiletine concentrations in serum (total and unbound) and saliva with time are shown in Fig. 1. In all subjects, the saliva drug level was always higher than the serum level and its time-course appeared to be almost parallel with that of the serum. Pharmacokinetic parameters for mexiletine in serum and saliva are summarized in Table 1. In all of the parameters estimated from the serum (total and unbound) and saliva data, there was considerable inter-subject variation. As shown by values for t_{max} for serum total, serum unbound and saliva samples, there was no time-lag between the peaks in serum and saliva in the individual subjects. However, first-order absorption rate constant or hybrid parameters, α and β, tended to have sample-type specific differences. The elimination half-life ($t_{\frac{1}{2}\beta}$) estimated from the saliva data tended to be longer than that from the serum data.

Correlation between serum and saliva drug concentrations. From some sample-type specific differences described above, examination of the relationship between serum and saliva drug levels was limited to the data in the terminal elimination phase during 4 to 24 h. Table 2 summarizes the regression equations for the mexiletine levels in saliva against the levels in serum in each

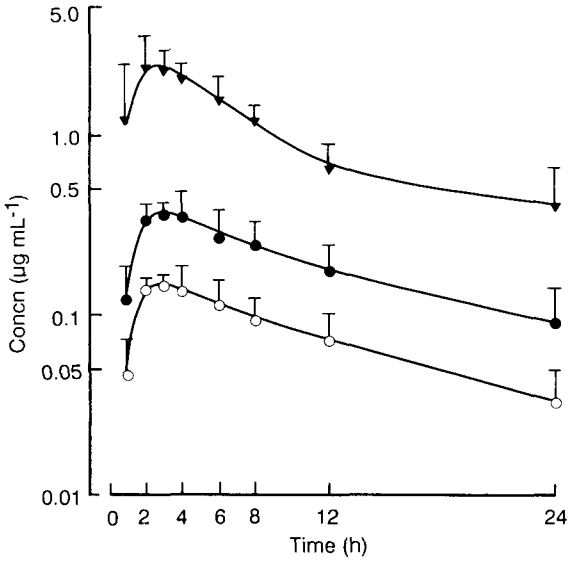

FIG. 1. Serum (total, ●; unbound, ○) and saliva (▼) levels of mexiletine after a single oral administration of two Mexitil capsules. Each point and vertical bar represent the mean and s.d. of five subjects. The lines represent the computer-fitted curves for the mean data [weight(i) = 1/Ci, where C is the drug concentration].

Table 1. Pharmacokinetic parameters for serum and saliva mexiletine after a single oral dose of a Mexitil capsule.

Parameters	Serum total	Serum unbound	Saliva
α (h^{-1})	0·895 ± 0·378*	0·667 ± 0·742	0·308 ± 0·149
β (h^{-1})	0·065 ± 0·022*	0·063 ± 0·028	0·037 ± 0·011
ka (h^{-1})	0·970 ± 0·375	2·72 ± 2·54	3·22 ± 4·56
t_{max} (h)	2·26 ± 1·57	1·72 ± 1·14	1·80 ± 1·22
$t_{\frac{1}{2}\beta}$ (h)	12·4 ± 6·40	13·4 ± 7·11	20·0 ± 5·83
$t_{\frac{1}{2}}$ka (h)	0·869 ± 0·525	0·577 ± 0·582	0·706 ± 0·701

Each value represents the mean ± s.d. of five subjects. Estimated by program MULTI [weight(i) = 1/Ci]. *There is a significant difference from saliva ($P < 0·05$).

subject. Although there were significant correlations between them ($P < 0·001$–$0·05$) in all subjects except No. 3, there was a large variation in the slope of the individual regression equations. Similar results were obtained for the serum unbound levels. Pooled data from all subjects also showed correlation between serum and saliva levels [total, $y = 3·95x + 0·28$, $r = 0·753$ ($P < 0·001$), $n = 25$; unbound, $y = 10·5x + 0·2$, $r = 0·786$ ($P < 0·001$), $n = 25$].

Comparison of observed and calculated saliva/serum ratios. Estimation of the saliva/serum ratio was made according to the following equation (Matin et al 1974):

$$\frac{C_{saliva}}{C_{serum}} = \frac{1 + 10^{pKa-pH_{saliva}}}{1 + 10^{pKa-pH_{serum}}} \cdot \frac{f_{serum}}{f_{saliva}}$$

The pKa value of mexiletine is 9·06; pH_{saliva} and f_{serum} were determined in each sample, pH_{serum} was assumed to be 7·40 and f_{saliva} was assumed to be 1·0. In our subjects, pH_{saliva} ranged from 5·95 to 7·09 and f_{serum} from 0·304 to 0·612. Table 2 also summarizes the observed and calculated saliva/serum ratios in four subjects. Although the observed saliva/serum ratio varied markedly among subjects, a consistent ratio was obtained in each individual.

Table 2. Regression equation for the mexiletine levels in saliva against the serum levels and comparison of the observed saliva/serum ratio with the calculated ratio in each subject given two Mexitil capsules.

		Saliva/serum ratio (mean ± s.d.)	
Subject	Regression equation[a]	Observed	Calculated[b]
1	$y = 9·89x - 0·19$ ($r = 0·957$)[c]	7·9 ± 1·9	4·2 ± 2·5
2	$y = 8·25x - 0·19$ ($r = 0·978$)[d]	6·8 ± 1·3	6·2 ± 3·1
3	$y = 8·69x - 0·68$ ($r = 0·809$)	—	—
4	$y = 4·44x - 0·14$ ($r = 0·993$)[e]	3·7 ± 0·4	1·0 ± 0·2
5	$y = 3·96x - 0·14$ ($r = 0·977$)[d]	3·5 ± 0·7	3·7 ± 1·6

[a] Regression analysis for $n = 5$ in post-absorption phase. y = saliva mexiletine; x = serum mexiletine; r = correlation coefficient. [b] By Matin's equation. [c] $P < 0·05$; [d] $P < 0·01$; [e] $P < 0·001$.

Discussion

Mexiletine concentrations in saliva were always higher than those in the serum of all subjects (Fig. 1, Table 2). These results are similar to those obtained by Beckett & Chidomere (1977) who reported higher drug levels in saliva than in blood plasma of normal subjects. This could prove useful during clinical trials, utilizing mexiletine levels in saliva for the drug's therapeutic monitoring, because saliva samples allow lower assay limits than do serum or plasma. For other drugs possessing higher saliva/serum (or saliva/plasma) ratios than unity, such as lithium (Groth et al 1974), metoprorol (Dawes et al 1978) and procainamide (Galeazzi et al 1976), it has been suggested that there may be some active transport mechanisms of the drug from blood into saliva in man.

An almost consistent correlation between saliva and serum mexiletine concentrations (or saliva/serum ratio) was obtained in each individual (Table 2). However, the estimation of serum concentrations from the saliva levels according to Matin's equation failed in two subjects. In these subjects, Matin's equation overestimated the serum levels. If there is an active transport mechanism for mexiletine excretion into the saliva, Matin's equation does not hold. However, from the above consistency, it was suggested that we could predict steady-state mexiletine levels in serum even from the saliva drug levels of patients in whom Matin's equation does not hold by using one-point, reliable data for saliva/serum ratio after the preceding single dose.

In conclusion, monitoring of mexiletine by using saliva samples is a useful, reliable and non-invasive way to individualize the dosage regimens for drug therapy.

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The lipid peroxidation product 4-hydroxy-2,3-*trans*-1 nonenal decreases rat intestinal smooth muscle function in-vitro by alkylation of sulphydryl groups

A. VAN DER VLIET, E. M. VAN DER AAR, A. BAST, *Department of Pharmacochemistry, Faculty of Chemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands*

Abstract—The effects of the lipid peroxidation product 4-hydroxy-2,3-*trans*-1 nonenal (HNE) on intestinal smooth muscle function have been studied. Exposure of rat isolated small intestinal segments to HNE (0·1–0·5 mM) led to decreased muscarinic and β-adrenergic responses. The maximal response to the muscarinic agonist methacholine and its pEC50 decreased in a dose dependent manner. The response to the β-adrenoceptor agonist isoprenaline was affected in a similar manner, but at slightly higher concentrations of HNE. As HNE has been described to be sulphydryl-reactive these effects were compared with the effects of the sulphydryl-reactive agent *N*-ethylmaleimide (NEM). Incubation of intestinal segments with NEM had similar effects on pharmacological responses to methacholine, indicating that the effects of HNE like that of NEM are likely to be caused by alkylation of sulphydryl groups. Dithiothreitol, a compound which reduces oxidized sulphydryl groups, was unable to restore the effects of HNE or NEM, which suggests that the effects of HNE and NEM are irreversible.

Oxidative stress has been shown to alter smooth muscle function in the intestinal tract. Hydrogen peroxide (an important reactive oxygen metabolite which is formed after intestinal ischaemia and reperfusion or during inflammatory processes) or cumene hydroperoxide (a model compound which is used to induce lipid peroxidation) deteriorates receptor-dependent or -independent contraction of the smooth muscle (Van der Vliet et al 1989). The effect of cumene hydroperoxide has been shown to be related to the process of lipid peroxidation, since inhibitors of lipid peroxidation also prevent smooth muscle damage by cumene hydroperoxide (Van der Vliet et al 1990b). One major product of lipid peroxidation has been shown to be 4-hydroxy-2,3-*trans*-nonenal (HNE) (Benedetti et al 1980; Pryor & Porter 1990) and this compound has direct actions on smooth muscle (Van der Kraaij et al 1990) and is able to affect receptor responses (Leurs et al 1986; Haenen et al 1989). Therefore we have investigated the effects of HNE on receptor responses in the rat small intestinal smooth muscle.

Correspondence: A. Van der Vliet, Department of Pharmacochemistry, Faculty of Chemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

Materials and methods

Chemicals. Methacholine hydrochloride, (−)-isoprenaline hydrochloride, *N*-ethylmaleimide (NEM), dithiothreitol (DTT) and N^6-2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate (sodium salt) (dibutyryl-cAMP) were obtained from Sigma Chemical Co. (St Louis, USA). 4-Hydroxy-2,3-transnonenal (HNE) was synthesized in our laboratory as described earlier (Leurs et al 1986). All other chemicals were of reagent grade.

Pharmacological measurements. Male Wistar rats, 220–250 g (Harlan C.P.B., Zeist, The Netherlands), were killed by decapitation and the midpart of the small intestine was removed rapidly and rinsed with Krebs buffer. Segments of about 1·5 cm were mounted in 20 mL tissue baths and kept at 37°C in Krebs buffer which was continuously gassed with 95% O_2–5% CO_2 (pH = 7·4). After equilibration for 30 min concentration-response-curves for methacholine or isoprenaline were recorded by cumulative addition of the agonists (Van der Vliet et al 1989, 1990a). After construction of the control curve, the segments were exposed to various concentrations of HNE or NEM for 20 min, during which time the smooth muscle tension was recorded isotonically. After incubation with HNE or NEM and extensive washing (30 min, during which the buffer was refreshed three times), a second concentration-response-curve was measured. In some experiments 1 mM DDT was added after incubation of intestinal segments with HNE or NEM during the first 20 min of the washing period.

Calculation of the data and statistics. Activities of the agonists used are expressed as pEC50 (i.e. log EC50). Maximal responses of the small intestine to methacholine or (−)-isoprenaline after treatment with HNE or NEM were related to the maximal response before treatment. Time-matched control curves were recorded for statistical analysis of differences between treated and untreated segments, which were determined using Student's *t*-test. $P < 0.05$ was regarded as significant.

Results and discussion

At concentrations of 0·1–0·5 mM, HNE affects the basal motility of the small intestine, which is completely inhibited at higher