Mandibular and Parotid Salivary Levels of Indomethacin Following Intravenous Administration to Rabbits[3]

Jun Watanabe[1], Tsutomu Setoyama[1], Kikuo Iwamoto[1,2], Yayoi Hayashi[1], and Shoji Ozeki[1]

Received: September 10, 1984; accepted February 11, 1985.

Abstract: Indomethacin was measured in mandibular and parotid saliva, obtained from separate cannulas in the salivary ducts, after bolus intravenous administration (15 mg/kg) to male white rabbits that were stimulated for salivation with pilocarpine given subcutaneously. There was a significant correlation between each salivary drug concentration and plasma drug concentration. Saliva to plasma drug concentration ratio (S/P ratio) and pH were higher in mandibular saliva than in parotid saliva. These gland specific differences were in contrast with the previously reported differences in dogs. Matin's equation was found to predict approximately the mean observed S/P ratio of indomethacin for each saliva sample.

The salivary excretion of drugs has been the subject of several investigations during the past decade. The observation that drug levels in saliva are often proportional to their plasma levels has led to the suggestion that in pharmacokinetic studies and therapeutic drug monitoring saliva might be substituted for plasma (1, 2). The most distinct advantage is that saliva can be collected by non-invasive techniques after stimulation of salivary secretion by chewing on semi-solid materials or applying gustatory chemicals such as citric acid onto the tongue.

If saliva is intended to be used as a sample in drug monitoring, the essential prerequisite is the existence of a consistent correlation between drug concentrations in plasma and those in saliva over a broad concentration range. Evidence already exists that many drugs or chemicals are excreted into saliva by a simple diffusion process and that lipid solubility may be a determining factor of salivary excretion (3, 4). Matin et al. have subsequently proposed that the saliva over plasma concentration ratios (S/P ratios: R) for weak acidic or basic compounds can be predicted from a modified pH-partition hypothesis (5). For acidic compounds, the following equation can be applied:

$$R = \frac{1 + 10^{pH_S - pKa}}{1 + 10^{pH_P - pKa}} \cdot \frac{f_P}{f_S}$$

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.

Although our previous reports have demonstrated significant gland specific differences and effects of salivary protein binding and salivary flow rate on the S/P ratio and/or salivary clearance of various compounds in dogs, results that suggested caution in the clinical usage of drug levels in whole saliva (6, 7, 8, 9), experimental animal species other than dogs have not been utilized at all for these purposes. The present work is, therefore, designed to establish the experimental techniques to separately collect mandibular and parotid saliva samples in rabbits, to verify Matin's equation for salivary drug excretion using indomethacin as a model drug, to test for gland specific differences in the S/P ratio, and to compare these results with our previous results for this drug in dogs (6).

Materials and Methods

Fistulation of Salivary Ducts in Rabbits

Male white rabbit (3.0–4.0 kg) were anesthetized with pentobarbital (25 mg/kg, i. v.) for surgery on the day before experiments. Parotid (Pr) duct fistulation was performed by modifying the methods reported by Kimura (10) and Kawasaki and Yasuda (11). As illustrated in Fig. 1 a, an incision of approximately 4 cm was made on the cheek skin 3 cm below the lower eye-ground just along the extrapolated line from the mouth corner. After the parotid duct was exposed along with the facial nerve by careful dissection of the subcutaneous connective tissue (Fig. 1 b), it was cannulated with PE-50 tubing (i. d. 0.58 mm; o. d. 0.965 mm, Clay Adams). The subcutaneous portion of the cannula was prevented from being pulled out from the duct by means of a small convex piece of double-layered silicone tubing which was tightly affixed around the PE-50 tubing. The distal portion of the cannula was then passed outside through the cheek skin, and the incised portion was then tied off (Fig. 1 c).

There has been no previous report describing the operation procedure for mandibular (M) duct fistulation. A dissection approximately 2 cm long from one corner of the mouth was made with a thermoknife (E-23, Natsume Seisakusho Co.), as shown in Fig. 1 d, and a cheek distender of solid but slightly flexible wire (o. d. 1.0 mm, Ω-shaped) was then applied at the upper and lower front teeth to facilitate the cannulation. PE-50 tubing (2 cm length) was inserted from one orifice into the mandibular duct (Fig. 1 e) with the aid of a stainless-steel wire guide (o. d. 0.35 mm; length 10 cm). After convex silicone tubing pieces were affixed in a way similar to that used for the Pr cannula, the distal portion of the PE-50 tubing was passed through the submandibular skin to the outside, and the dissected mouth corner was tied off (Fig. 1 f).

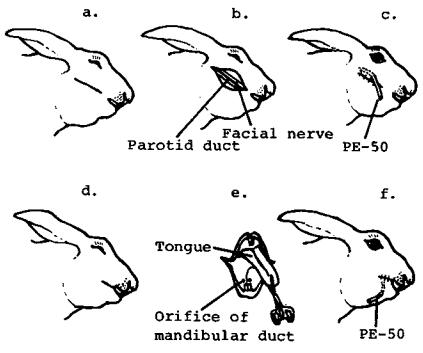

Fig. 1 Fistulation method for parotid (a, b, c) and mandibular (d, e, f) salivary ducts in rabbits.

[1] Department of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Nagoya City University, 3–1 Tanabe-dori, Mizuho-ku, Nagoya 467, Japan
[2] Correspondence
[3] This paper constitutes Part V of the series entitled "Salivary excretion of drugs"

After recovery from anesthesia, salivation was stimulated once by applying a few drops of 10% citric acid solution onto the tongue (6, 7) to ensure the proper fistulation for both salivary (Pr and M) ducts for periodical collection of saliva samples.

Collection of Plasma and Saliva Samples Following Intravenous Administration of Indomethacin

Blood samples were obtained from the marginal ear vein into heparinized syringes, while each saliva sample was collected through an PE-50 extension tubing (6 to 8 cm) into the bottom of small sample tube (2 ml in size, Niplon Co.) containing 200 µl liquid paraffin to avoid any exposure of saliva samples to the atmosphere (6, 7, 8, 9). Salivation was stimulated with 0.1 ml/kg of 1% pilocarpine hydrochloride solution which was injected subcutaneously every 30 min. After collecting reference blood and saliva samples, indomethacin (IM, Nippon Merck-Banyu Co.) dissolved in 0.6% sodium carbonate (6) was administered intravenously at 15 mg/kg via a marginal ear vein. Saliva samples (0.6 to 1.0 ml) were periodically collected for 2 to 10 min, and blood samples (2.2 ml) were simultaneously collected at the midpoint of the saliva collection intervals. Salivary flow was estimated from the sample weight assuming that its specific gravity was 1.00 (7).

Binding of Indomethacin to Plasma and Saliva Proteins

One ml samples of plasma and saliva were spiked with 5 to 100 µg and 1 to 10 µg indomethacin, respectively, and dialyzed against 2 ml pH 7.4 isotonic phosphate buffer solution using seamless cellulose tubing (Type 8/32, Visking Co.) at 37°C. In this dialysis system, equilibrium was attained within 10 h.

Analytical Procedures

After immediate centrifugation (3000 rpm, 10 min) of the blood samples the indomethacin concentration in plasma (1 ml) was determined by a previously described method (6). After measuring the weight and pH of saliva samples, salivary indomethacin concentrations were similarly determined except that the saliva volume was only 0.5 ml. Drug concentrations in both inner and outer phases after the equilibrium dialysis were also determined by the same method (6). Fluorometric measurement was performed at λ_{ex}= 283 nm and λ_{em}= 378 nm with a 1 ppm quinine sulfate solution as the instrument (Shimadzu RF-510) standard.

Protein levels in plasma and saliva were determined by Lowry's method (12) with bovine plasma albumin (Fraction V, Armour Pharmaceutical Co.) as a standard.

Data Analysis and Statistical Evaluation

Both plasma and salivary indomethacin concentration-time curves were analyzed according to the least-squares regression analysis program MULTI (13) for a bi-exponential decline expressed as $C = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$, where C is the drug concentration and A, B, α and β are hybrid parameters. Criteria for both convergence and best fit were the same as reported previously (13). Differences in salivary pH values and saliva to plasma concentration ratios (S/P ratio) were evaluated by Student's t-test after determining that each data population was normally distributed.

Results and Discussion

Plasma and Saliva Indomethacin Concentration-Time Profiles

Figure 2 represents indomethacin concentration-time profiles for plasma and saliva after intravenous administration to four rabbits. Only plasma concentration data could be analyzed by the bi-exponential equation as indicated with the computer-fitted solid line (Fig. 2). Mandibular salivary drug levels were always higher than the parotid levels. Although both salivary indomethacin levels (M and Pr) appeared to decline almost in parallel with the plasma level, neither the present nor an alternative least-squares regression method for the bi-exponential decline could analyze both sets of the saliva level-time data, probably because of relatively large fluctuations or inter-individual variations.

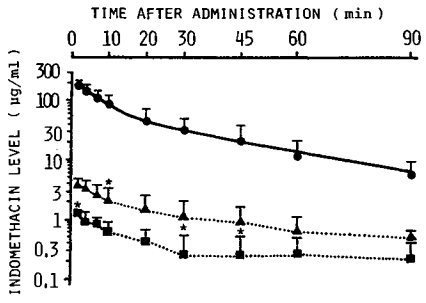

Fig. 2 Plasma (●) and saliva (M: ▲, Pr: ■) indomethacin concentrations following bolus intravenous administration of 15 mg/kg to four rabbits. Salivation was stimulated with 0.1 ml/kg of a 1% pilocarpine hydrochloride solution injected subcutaneously every 30 min. Each point with vertical bars represents the mean with S.D. from three(*) to four rabbits. The solid line was computer-fitted for all individual data points (n=36) with weighting of $1/C^2$. Estimated parameters are as follows: A = 162±34 µg/ml, B = 65.1±16.9 µg/ml, α = 0.103 ±0.025 min^{-1}, β = 0.0161±0.0071 min^{-1}.

Correlation Between Saliva and Plasma Indomethacin Concentrations

There were relatively scattered but statistically significant ($p<0.01$) correlations between each saliva and plasma indomethacin concentrations (Fig. 3).

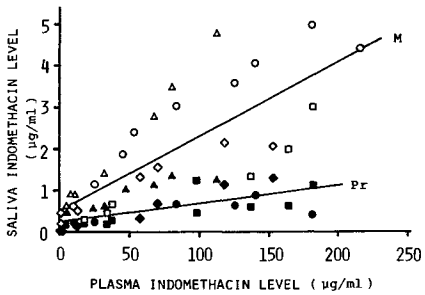

Fig. 3 Correlation between saliva and plasma indomethacin concentrations following bolus intravenous administration of 15 mg/kg to four rabbits. Each symbol (Pr: ●, ▲, ■, ◆; M: ○, △, □, ◇) represents the result from an individual rabbit. Linear regression line was given as Y = 0.00433X +0.257 for Pr data (r = 0.639, n = 33, $p<0.01$) and Y = 0.0177X+0.533 for M data (r = 0.799, n = 35, $p<0.01$).

Table I summarizes the mean values for S/P ratios and some biological data characteristic of saliva samples. In rabbits stimulated for salivation with pilocarpine, the S/P ratio was significantly ($p<0.05$) larger in M saliva than in Pr saliva, contrary to the results in dogs that were stimulated with citric acid; in the dog experiments mandibular-sublingual saliva (MS) samples were collected instead of M saliva alone (6). The gland differences in the S/P ratio for indomethacin in rabbits and the species differences may result from gland specificity and species-dependent differences in salivary pH. In rabbits, however, the S/P ratios for both Pr and M salivas were quite variable.

Table I. Comparison of S/P Ratio, Salivary pH and Protein Concentration following Intravenous Administration of Indomethacin to Four Rabbits (15 mg/kg) and to Six Dogs (20 mg/kg).

		Rabbits[a]		Dogs[b]	
S/P Ratio	Pr	0.0151 ± 0.0199[c]	(33)[d]	0.074 ± 0.034	(60)
	M or MS[e]	0.0608 ± 0.102	(35)	0.044 ± 0.024	(55)
Salivary pH	Pr	7.28 ± 0.20	(34)	8.16 ± 0.21	(32)
	M or MS	7.81 ± 0.15	(31)	7.82 ± 0.20	(36)
Protein Concn.	Pr	4.10 ± 1.75	(24)	2.2 ± 0.5	(4)
(μg/ml)	M or MS	3.33 ± 1.31	(19)	1.7 ± 0.1	(4)

[a] Stimulated with 1 % pilocarpine
[b] Stimulated with 10 % citric acid [from reference (6)]
[c] S.D.
[d] Number of data points
[e] Mandibular-sublingual saliva in dogs

Prediction of S/P Ratio by Matins Equation

Since indomethacin is a weakly acidic drug, Matin's equation may serve to predict its S/P ratio. If one takes the mean values for observed pH_S(pH_{Pr}= 7.28, pH_M= 7.81), f_P(= 0.0154) and f_S(f_{Pr}= 0.917, f_M= 0.898) as well as pH_P(= 7.4) into account, the predicted S/P ratios (Pr = 0.0127, M = 0.0439) were fairly close to the corresponding mean observed ratios. It is thus suggested that Matin's equation can predict fairly accurately at least the mean S/P ratio for indomethacin in rabbits, which is in contrast to the poor prediction found in dogs (6). However, the prediction by Matin's equation for the individual S/P ratios for each M and Pr saliva was relatively poor.

Salivary Clearance of Indomethacin

Salivary clearance has been introduced very recently to describe kinetics of salivary excretion of drugs or chemicals (8, 9, 14, 15), and it is defined as the product of S/P ratio and salivary flow (8,14). The mean salivary clearance of indomethacin in M saliva (0.900±1.17 μl/min/kg) tended to be larger than that in Pr saliva (0.490±0.412 μl/min/kg). Large fluctuations in these clearance values might be partially due to relatively large fluctuation in the observed S/P ratio as discussed above. If one doubles the sum of the mean salivary clearance values for Pr and M saliva because both glands exist in pair, the total salivary clearance can be calculated to be 2.78 μl/min/kg under continuous stimulation of salivation. This value corresponds to only 1 % of the total body clearance of indomethacin (2.67 ml/min/kg) in the same rabbits, suggesting that salivary excretion does not play an important role in the overall elimination of indomethacin from the body, as opposed to that of urea or phenobarbital in dogs (more than 15 % of the total body clearance) (8).

In conclusion, the present technique to separately collect parotid and mandibular saliva samples in rabbits can serve to study salivary drug excretion before initiating any clinical salivary drug monitoring.

References

(1) Dvorchik, B. H., Vesell, E. S. (1976) Clin. Chem. 22, 868–878.
(2) Horning, M. G., Brown, L., Mowlin, J., Lertratanangkoon, K., Kellaway, P., Zion, T. E. (1977) Clin. Chem. 23, 157–164.
(3) Rasmussen, F. (1964) Acta Pharmacol. et Toxicol. 21, 11–19.
(4) Gruneisen, A., Witzgall, H. (1974) Eur. J. Clin. Pharmacol. 7, 77–79.
(5) Matin, S. B., Wan, S. H., Karam, J. H. (1974) Clin. Pharmacol. Ther. 16, 1052–1058.
(6) Watanabe, J., Urasaki, Y., Nakase, Y., Ueda, H., Iwamoto, K., Ozeki, S. (1981) J. Pharm. Dyn, 4, 336–344.
(7) Watanabe, J., Nakase, Y., Urasaki, Y., Hayashi, Y., Iwamoto, K., Ozeki, S. (1981) J. Pharm. Dyn. 4, 968–977.
(8) Watanabe, J., Mizuno, S., Masuda, N., Hayashi, Y., Iwamoto, K., Hirate, J., Ozeki, S. (1984) J. Pharm. Dyn. 7, 294–303.
(9) Watanabe, J., Hayashi, Y., Iwamoto, K., Ozeki, S. (1985) Chem. Pharm. Bull. 33, 1187–1194.
(10) Kimura, Y. (1957) Shika-gakuho 32, 35–37.
(11) Kawasaki, H., Yasuda, T. (1963) Shika-gakuho 37, 37–39.
(12) Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
(13) Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) J. Pharm. Dyn. 4, 879–885.
(14) Graham, G. G. (1982) Pharmacol. Ther. 18, 333–349.
(15) Zuidema, J., Van Ginneken, C.A.M. (1983) Pharm. Acta Helv. 58, 88–93.