EXCRETION OF INDOMETHACIN INTO SALIVA FOLLOWING INTRAVENOUS ADMINISTRATION TO DOGS*

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Parotid saliva (Pr) and mandibular-sublingual saliva (MS) were collected separately by means of permanent fistulae in order to investigate the excretion of indomethacin in saliva of dogs receiving a single intravenous dose of 20 mg/kg. Drops of citric acid solution were placed on the tongue to stimulate salivary secretion.

The concentrations of indomethacin both in saliva and plasma declined biexponentially with time. There was a good linear relationship between the drug concentration in each saliva and plasma. The Pr and MS levels were 7.4% and 4.4% of the plasma levels, respectively. Indomethacin concentrations in Pr were significantly higher than in MS (p < 0.05). The roles of salivary pH and salivary protein binding were discussed in respect to the mechanism of salivary excretion of the drug.

Keywords—drug monitoring; drug level in saliva; indomethacin; relationship between saliva and plasma drug concentrations; salivary fistula in dog; salivary excretion mechanism; salivary protein binding

With the development of clinical pharmacokinetics, it has been widely recognized that the determination of drug concentration in blood plasma or serum is often very important. However, monitoring blood drug levels requires much time and effort in addition to frequent collection of blood samples, and blood samples can not be obtained without a number of punctures which give much pain to patients. Therefore, rapid and non-invasive techniques for drug level monitoring have been sought recently.

It has been reported for many drugs that drug concentrations in saliva are often proportional to the concentrations in plasma and equal the free or protein-unbound concentrations in plasma.1-13 These observations have suggested that saliva might be substituted for plasma in drug level monitoring. Furthermore, drug concentrations in saliva may be therapeutically more meaningful than those in plasma, since the free fraction of the drug is directly available to produce pharmacological effects. From these points of view, the salivary excretion of drugs has attracted a renewed attention.

Matin et al.14 have indicated that for a weak acid, saliva/plasma concentration ratio (R) of the drug (S/P ratio) is described by the following equation,

\[
R = \frac{1 + 10^{(pH_s - pK_{a})}}{1 + 10^{(pH_{pl} - pK_{a})}} \cdot \frac{F_{pl}}{F_S}
\]

where \(pH_s\) = pH of saliva, \(pH_{pl}\) = pH of plasma, \(pK_a = pK_{a}\), value of the drug, \(F_{pl}\) = unbound fraction of the total drug concentration.

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in plasma, and $F_S = \text{unbound fraction of the total drug concentration in saliva}$. They have reported that $R$ value (S/P ratio) observed for tolbutamide ($pK_a = 1.4$)\textsuperscript{14} or salicylic acid ($pK_a = 2.75$)\textsuperscript{10} is in good agreement with the value predicted by using Eq. (1), when $pH_S = 6.5$ and $F_S = 1$. However, $pH_S$ value is practically variable, then variance of $R$ value may also be involved. McAuliffe et al.\textsuperscript{16} reported the increase of $R$ value of phenobarbital with the rise of salivary pH. Besides, it seems that the assumption where $F_S = 1$ should not be always correct, since parotid and mandibular-sublingual saliva differ markedly in their composition, and the relative amounts of saliva secreted by the different types of glands vary with the change of stimuli.\textsuperscript{17}

Therefore, more detailed investigation on the mechanism of salivary drug excretion may be necessary before saliva instead of plasma is used effectively to evaluate the drug level in the body.

In this paper, salivary fistulae were produced in dogs to collect parotid saliva (Pr) and mandibular-sublingual saliva (MS) separately, and the relationship between the concentrations of indomethacin in each saliva and plasma was studied after rapid intravenous administration of the drug. Furthermore, salivary pH and binding of indomethacin to salivary protein were measured to examine the applicability of Eq. (1) to the salivary excretion of this drug.

**EXPERIMENTAL**

**Materials** — Indomethacin was kindly supplied from Nippon Merck-Banyu Co., Ltd., Okazaki, Japan and Sumitomo Chemical Co., Ltd., Osaka, Japan and used without further purification. Paraffin liquid for measurement of salivary pH and citric acid for salivary stimuli were of reagent grade. All other chemicals used were of analytical grade.

**Animals** — Adult male mongrel dogs (body weight 11, 13 kg) or beagle dogs (body weight 10, 10, 11, 11 kg) having both permanent parotid and mandibular-sublingual salivary fistulae were used for the experiments without fasting at least 2 weeks after the operation to form fistulae.

*The Operation to form Fistulae in the Salivary Ducts of Dogs* — To obtain a pure secretion from each gland separately Pavlov has developed a method of producing chronic fistulae in the ducts of the salivary glands.\textsuperscript{18–20} The parotid duct

![Diagram of salivary glands](image)

**FIG. 1. Dog with Fistula of the Parotid or Mandibular-sublingual Gland**

**Dental formula:** $I \frac{3}{3} C \frac{1}{1} P \frac{4}{4} M \frac{2}{3}$

![Diagram of saliva collection](image)

**FIG. 2. Devices for Collection of Saliva with (c, d) or without Liquid Paraffin (a, b)**

(a), (c): For parotid saliva.

(b), (d): For mandibular-sublingual saliva.
opens into the buccal cavity at the anterior end of a blunt ridge of mucosa by a small papilla. This is located opposite to the posterior margin of the fourth upper premolar of carnassial tooth. The sublingual duct is closely related to the mandibular duct. These ducts open on a small sublingual papilla, which is located lateral to the anterior end of the mandible. The ducts of the two glands merge, forming a common opening. The operation for producing fistulae in these salivary ducts of dogs was performed as follows. Dogs were anesthetized with 25 mg/kg of sodium pentobarbital administered into cephalic vein. Five cm of probe (24 gauge stainless steel wire) was inserted into parotid duct or mandibular-sublingual saliva duct gently but firmly along its course from the orifice to the gland. A piece of the mucous membrane of the mouth, where the opening of the duct of the parotid or mandibular-sublingual gland was located, was excised in a circle of about 1 cm in diameter. The duct and the piece of mucosa were separated from the surrounding tissues with great care to avoid an injury to the duct. The separated disk of mucosa were brought to the outside through a puncture in the cheek or in the floor of the oral cavity, and sewn to the skin as shown in Fig. 1. The piece of mucosa grew gradually into a part of the skin on the cheek or the chin.

Collection of Saliva — Devices for collection of saliva were made with polyethylene tubing (i.d. 6 mm). The devices were attached to the skin to cover the opening of the duct using Aron Alpha (Touagousei Co.) as shown in Fig. 2 schematically. Two kinds of saliva were introduced directly into test tubes or led through Tygon tubings (i.d. 1/16 inch) under the liquid paraffin layer in test tubes. The liquid paraffin was used for avoiding the exposure of samples to the atmosphere in order to obtain the exact pH values. Usually, the salivary flow in human is stimulated by having subjects chew a piece of paraffin, parafilm, or teflon, or stimulated with citric acid or a subcutaneous injection of 8—12 mg pilocarpine. It has also been known that pilocarpine (s.c., 0.05 mg/kg) is used to stimulate the salivary flow in dogs. A subcutaneous injection of 0.05 mg/kg pilocarpine was at first tried to stimulate the salivary secretion in this study. Under the condition, the mean flow rates were 0.04 ml/min/kg for parotid saliva (Pr) and 0.06 ml/min/kg for mandibular-sublingual saliva (MS). When 0.4 ml of 10% citric acid solution was dropped onto the tongue of a dog, the salivary secretion continued for about 3 min and the mean flow rates for Pr and MS were 0.11 and 0.12 ml/min/kg, respectively. Since the secretion rates by citric acid stimuli were about two times as large as those by pilocarpine stimuli for both types of saliva, droppings of about 0.5 ml of 10% citric acid solution onto the tongue several times every 30 sec were employed as stimuli to collect saliva in this

<table>
<thead>
<tr>
<th>TABLE I. Pharmacokinetic Parameters for Indomethacin following Bolus Intravenous Administration of 20 mg/kg in Dogs</th>
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<tr>
<td>A, μg/ml</td>
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<tr>
<td>B, μg/ml</td>
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<tr>
<td>α, min⁻¹</td>
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<tr>
<td>β, min⁻¹</td>
</tr>
<tr>
<td>τ₁ α, min</td>
</tr>
<tr>
<td>τ₂ β, min</td>
</tr>
</tbody>
</table>

a) Pr: Parotid saliva, b) MS: Mandibular-sublingual saliva, c) The number of input data, d) Standard error.
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experiment.

**Measurement of Salivary pH** — Salivary pH increases gradually by the exposure of the saliva to the atmosphere. Therefore, the saliva samples were collected under the liquid paraffin layer and the salivary pH was measured with the combined electrode immersed through the layer.

**Drug Administration and Sampling of Biological Fluids** — Twenty mg/kg of indomethacin was administered intravenously to a dog through the cephalic vein as the aqueous solution (20 mg/ml) of 0.6% sodium carbonate. Blood and saliva samples were taken simultaneously, when the mid time of a sample collection period was represented as the sampling time. The plasma was obtained by centrifugation of blood at 3000 rpm for 15 min.

**Measurement of Protein Binding of Indomethacin in Saliva** — An ultrafiltration method, similar to that described by Coolidge,²⁷ was used to measure the binding of indomethacin to saliva protein. Four ml of saliva sample was placed inside the sac of a Visking tube (Type 20/30, 25 cm, Visking Company), and was centrifuged at 1500 rpm for 5 min and then at 2500 rpm for 60 min. The free fraction of indomethacin was calculated by the following ratio: (indomethacin concentration in filtrate)/(the initial concentration in saliva sample). To detect the leakage of protein through the membrane, Lowry method²⁸ was applied to the filtrates, and the data from the filtrates with high protein levels were excluded from the binding data. The ultrafiltration using Diaflo membrane (Type XM50, Amicon) was also examined, but was found to be unsuccessful probably due to the adsorption of indomethacin to the apparatus.

**Determination of Indomethacin** — Indomethacin in dog plasma and saliva was determined by a modification of the method of Holt and Hawkins²⁹ Hucker et al.,³⁰ Moriyama et al.,³¹ and Hvidberg et al.³²) One ml of plasma or saliva was added to 2 ml of 1 M citrate buffer, pH 5.0 and 30 ml of heptane containing isooamylalcohol (3% for saliva; 1.7% for plasma). The mixture was shaken for 15 min and centrifuged at 3000 rpm for 15 min. Twenty-five ml of the heptane phase was transferred into a centrifuge tube containing 5 ml of 0.2 M citrate buffer, pH 5.0. The mixture was again shaken and centrifuged under the same condition as described above, and 20 ml of the heptane phase was transferred into another centrifuge tube containing 5 ml of Kolloff buffer, pH 11.6. After shaking and centrifuging, the upper heptane layer was discarded by aspiration. Four ml of the lower aqueous layer was transferred to another glass stoppered tube, and then incubated for 30 min at 50º, followed by cooling for 5 min in a water bath. The fluorescence of the aqueous phase was measured in a Hitachi 204 spectrofluorophotometer (excitation wavelength: 305 nm, emission wavelength: 375 nm). Quinine sulfate solution in 0.1 N sulfuric acid was used as the instrument standard (excitation wavelength: 370 nm, emission wavelength: 450 nm).

**RESULTS AND DISCUSSION**

**Relationship between Indomethacin Concentration in Plasma and Saliva**

Indomethacin concentrations in plasma (P1), parotid saliva (Pr), and mandibular-sublingual saliva (MS) were measured following the bolus intravenous administration in dogs, and the results obtained are shown in Fig. 3. The concentrations both in Pr and MS were considerably lower than those in plasma, and Pr show rather higher indomethacin levels than MS. However, all the drug concentrations in those three biological fluids decreased biexponentially with time. Plasma and saliva levels of indomethacin were analyzed by the following equation, \( C = A e^{\alpha t} + B e^{\beta t} \), where \( C \) is the drug concentration and \( A, B, \alpha, \beta \) are hybrid parameters. These pharmacokinetic parameters were estimated by least-squares fitting³³ using a digital computer, HITAC 5020 (Hitachi Ltd., Kanagawa, Japan). Each weight of 1, \( 1/C \) and \( 1/C^2 \) was used in the estimation. The most probable regression curves calculated are shown by the solid lines in Fig. 3, and estimated parameter values are summarized in Table I. The values for
α from plasma, Pr, and MS levels are very close to one another. Furthermore, the significant difference is not found between the β value from plasma and that from MS (p > 0.2), though the β value from Pr took a rather higher value than that from plasma (p < 0.001). It might be necessary to develop a more sensitive analytical method of indomethacin especially to determine such low levels in saliva as in the β phase more accurately, since the coefficient of variation for indomethacin levels of saliva in β phase was higher than that in α phase.

To examine the correlation between indomethacin concentrations in saliva and plasma, the scatter diagrams were drawn and the coefficients of correlation were calculated. A fairly good linear correlation was observed between the drug concentrations in parotid saliva and plasma as shown in Fig. 4, and the coefficient of correlation (r) was 0.91. The similar relationship was also found between the drug concentrations in MS and plasma, and the r for this case was 0.94 as shown in Fig. 5. Mean values for saliva/plasma indomethacin concentration ratio (S/P ratio) were 0.074 and 0.044 for Pr and MS, respectively.

**Comparison of Indomethacin Levels in Parotid and Mandibular-sublingual Saliva**

A comparison of indomethacin concentrations in parotid saliva (Pr) with those in mandibular-sublingual saliva (MS) was carried out. It appeared that there was a good correlation between MS and Pr as shown in Fig. 6, and the coefficient of correlation (r) took the value of 0.94. The mean MS/Pr ratio (± S.D.) was 0.64 (± 0.32) These results indicate that indomethacin concentration in Pr is significantly higher than that in MS (p < 0.05) in dogs. Tsujimoto et al. reported that the concentrations of unchanged nicotine in mandibular saliva were higher than those in parotid saliva both in dogs and monkeys. These differences of salivary excretion of drugs between different glands may cause the S/P ratio to fluctuate under the condition, when whole or mixed saliva is used as the sample. However, very little has been known on the mechanism of salivary excretions of drugs from different glands.

In this paper, binding of indomethacin to saliva constituents and salivary pH were investigated to elucidate these different salivary excretions of the drug.

![FIG. 3. Plasma and Saliva Levels following Bolus Intravenous Administration of 20 mg/kg of Indomethacin in Six Dogs](image)

○ : Plasma, △ : parotid saliva, ◇ : mandibular-sublingual saliva. Each point and vertical bar indicate the mean value and the standard deviation, respectively. The solid curves show the computer-fitted curves (weight (l) = 1/C² for plasma and parotid saliva, weight (l) = 1 for mandibular-sublingual saliva, where C is the indomethacin concentration).

![FIG. 4. Relationship between Indomethacin Concentration in Plasma and Parotid Saliva in Dogs](image)
**Binding of Indomethacin to Saliva Constituents**

Experimental data of indomethacin binding to saliva constituents, probably salivary protein, are shown in Table II. Also included in the table are values of the protein concentrations in parotid saliva (Pr) or mandibular-sublingual saliva (MS), which were determined by Lowry method.²⁸

The ultraltrate/saliva concentration ratio of the drug for Pr was rather smaller than that for MS, indicating that indomethacin binding to protein was somewhat larger in Pr than in MS. In other words, Pr may have smaller $F_s$ values in Eq. (1) than MS for this drug. Since the smaller $F_s$ value gives the larger $R$ value, i.e., the larger S/P ratio according to Eq. (1), those salivary protein binding data in Table II are consistent qualitatively with the evidence that indicated higher concentrations of the drug in Pr than MS as shown in Fig. 6.

However, a more detailed investigation on salivary protein binding would be necessary to relate the binding data with S/P ratio quantitatively, since salivary protein concentrations observed were considerably lower than those in blood plasma (about 40 mg/mL).

**Salivary pH and Salivary Plasma Indomethacin Concentration Ratio (S/P Ratio)**

Another factor, which may affect salivary excretion of drugs, is pH of saliva (pH₆), since blood plasma pH (pH₆) varies within a very nar-

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**FIG. 5. Relationship between Indomethacin Concentration in Plasma and Mandibular-sublingual Saliva in Dogs**

**FIG. 6. Relationship between Indomethacin Concentration in Parotid Saliva and Mandibular-sublingual Saliva in Dogs**

**TABLE II. Indomethacin Protein Binding in Parotid Saliva and Mandibular-sublingual Saliva**

<table>
<thead>
<tr>
<th>Kind of saliva</th>
<th>Ratio of concentration $\left( \frac{\text{ultraltrate}}{\text{saliva}} \right)$</th>
<th>Protein concentration $^a$ (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parotid saliva</td>
<td>$0.653 \pm 0.064 \quad (n = 6)^c$</td>
<td>$2.2 \pm 0.5 \quad (n = 4)$</td>
</tr>
<tr>
<td>Mandibular-sublingual saliva</td>
<td>$0.751 \pm 0.024 \quad (n = 4)$</td>
<td>$1.7 \pm 0.1 \quad (n = 4)$</td>
</tr>
</tbody>
</table>

$^a$ Determined by the method of Lowry et al. $^{28}$

$^b$ Mean value ± standard deviation.

$^c$ The number of saliva samples collected from two dogs.
row range and then probably has little influence on the saliva/plasma drug concentration ratio (S/P ratio). Matin et al. used a constant pH of 6.5 as pH₉ in their calculation. However, salivary pH seems to be variable. In this study, it was observed that the mean value of pHₚ₉ (pH of parotid saliva) was 8.16 (±0.21:S.D., n = 32) and that of pH₉₉ (pH of mandibular-sublingual saliva) was 7.82 (±0.20:S.D., n = 36) in dogs. Using the average pH values, R values or S/P ratios were calculated according to Eq. (1). The results together with the observed values are shown in Table III. The calculated S/P ratio for parotid saliva (Cₚ₉/Cₚ₉) was higher than that for mandibular-sublingual saliva (C₉₉/Cₚ₉), and the tendency was consistent with the observed values.

However, the calculated concentration ratio of both kinds of saliva, C₉₉/Cₚ₉, was lower than the observed one, though the calculation was done assuming no protein binding of the drug in both Pr and MS, i.e., Fₚ₉ = 1 and F₉₉ = 1 as shown in the footnote of Table III. If a smaller value for Fₚ₉ was employed in the calculation according to the result in Table II, C₉₉/Cₚ₉ would take a still

### Table III. Indomethacin Saliva/Plasma Concentration Ratio

<table>
<thead>
<tr>
<th></th>
<th>Cₚ₉/Cₚ₉</th>
<th>C₉₉/Cₚ₉</th>
<th>C₉₉/Cₚ₉</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observed</strong></td>
<td>0.074±0.034&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.044±0.024&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.64±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Calculated</strong></td>
<td>0.115</td>
<td>0.053</td>
<td>0.46</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cₚ₉: the drug concentration in plasma, Cₚ₉: the drug concentration in parotid saliva, C₉₉: the drug concentration in mandibular-sublingual saliva.

<sup>b</sup> Eq. (1) in text was used, where pKₐ = 4.5, pHₚ₉ = 7.4, Fₚ₉ = 0.02 (from ref. 32), Fₚ₉ = 1, and F₉₉ = 1.

<sup>c</sup> Mean value ± standard deviation.

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**FIG. 7. Relationship between Observed Ratio and Calculated Ratio of Saliva Indomethacin Concentration to Plasma Concentration**

pHₚ₉: 8.16±0.21 (n=32), pH₉₉: 7.82±0.20 (n=36). Calculated saliva/plasma (S/P) indomethacin concentration ratio: S/P = (1 + 10<sup>pH₋pKₐ</sup>)/(1 + 10<sup>pH₋pKₐ</sup> × Fₚ₉/FS × Pr × (1 + 10<sup>pH₋4.5</sup>)/(1 + 10<sup>7.4−4.5</sup>) × 0.02/1, MS: (1 + 10<sup>pH₉₉₋4.5</sup>)/(1 + 10<sup>7.4−4.5</sup>) × 0.02/1.
lower value than the observed one. Furthermore, the S/P ratio was calculated by Eq. (1) for each pH value observed in each saliva sample, and then the correlation between the calculated S/P ratios and the observed ones was examined. The scatter diagrams for Pr and MS are widely dispersed as shown in Fig. 7. There were no significant correlations between the calculated and observed S/P ratio both in Pr and MS. It might be necessary to assume the intrinsic or effective pH values at the site of saliva secretion as suggested by Koup et al. 35 Otherwise, the protein concentration might be variable among saliva samples, and the fraction of unbound indomethacin in saliva (F) might fluctuate more than expected.

In any case, this study could not demonstrate complete availability of Eq. (1), the equation of Matin et al. For more detailed mechanism of drug excretion into saliva, drugs other than indomethacin are now being investigated using salivary fistulated dogs in this laboratory.

REFERENCES


25) S.A.Killmann, J.H.Thaysen: The permeability of the human parotid gland to a series of sulfonamide com-


