Salivary Excretion of 5-Fluorouracil. II. Fluctuation of Saliva/Plasma Concentration Ratio and Salivary Clearance during a Constant Rate Intravenous Infusion in Beagle Dogs*

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Salivary excretion of 5-fluorouracil (5-FU) was investigated during constant rate intravenous infusion (0.306 mg/kg/min) in three male beagle dogs. Parotid (Pr) and mandibular-sublingual (MS) saliva were collected separately by stimulating salivation with 10% citric acid.

After the start of 5-FU infusion, plasma and salivary 5-FU concentration increased rapidly to approach their steady state levels. There was a significant correlation between each saliva and plasma 5-FU concentration ($p < 0.01$). The saliva/plasma drug concentration ratio (S/P ratio) and salivary pH were significantly higher in Pr than in MS saliva ($p < 0.001$), similar to the results following bolus intravenous administration of 5-FU in beagle dogs. Both S/P ratio and salivary clearance increased with time before steady state. Thereafter, these values approached almost constant levels and their fluctuations became smaller than those following the bolus intravenous administration. These results showing S/P ratio and salivary clearance of 5-FU were affected by the plasma drug concentration, suggested the possibility that non-linear pharmacokinetics may be involved in the salivary excretion of 5-FU.

Keywords — 5-fluorouracil; salivary drug excretion; salivary drug concentration; salivary clearance; beagle dog

The salivary excretion of drugs has been studied extensively from clinical points of view, i.e. the possibility of substituting saliva for blood for therapeutic drug monitoring. 5-Fluorouracil (5-FU) is an antineoplastic agent extensively used in palliative and curative treatment of various solid tumors. Since plasma levels of 5-FU following the various rates and routes of administration often vary among patients, the drug is thought to belong to the group of drugs which require routine monitoring of the plasma levels to enhance their effectiveness and to minimize toxic side effects.

Celio et al. have reported that 5-FU was excreted in detectable amounts in parotid saliva following an intravenous administration to rats and humans and that there were huge interindividual and pronounced intraindividual differences in the saliva/plasma drug concentration ratios (S/P ratios). They concluded that the concentration of the drug in parotid saliva was not useful for predicting the plasma level of the free drug. In our previous report, following bolus intravenous administration (20 mg/kg) to beagle dogs, the S/P ratios showed large fluctuations in both parotid (Pr) and mandibular-sublingual (MS) saliva, and the salivary clearance in each saliva did not show a constant value with various plasma 5-FU concentrations.

In the present work, the magnitude of fluctuations of S/P ratio and salivary clearance was investigated in beagle dogs at a steady state plasma concentration achieved by constant rate intravenous infusion of 5-FU which was compared with that following a bolus intravenous administration to beagle dogs.

Experimental

Animals — Three male beagle dogs weighing 8.0—10.0 kg were used without fasting. All beagle dogs had permanent fistulae for collect-

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ing Pr saliva and MS saliva separately.

**Drug Administration and Collection of Plasma and Saliva Samples** — After collecting reference blood and saliva samples, 5-FU was administered as an intravenous infusion into the cephalic vein in a dose of 0.306 mg/kg/min. This infusion rate, $k_0$, was derived from the following equation which was aimed to provide a steady state drug concentration in plasma, $C_{ss}$, 10 $\mu$g/ml,

$$k_0 = C_{ss} \cdot V_d \cdot k_{el}$$

(1)

where the apparent volume of distribution of the drug, $V_d$, was set to be 0.351 l/kg, and the apparent first-order elimination rate constant, $k_{el}$, 0.0871 min$^{-1}$. The values used were obtained in the previous work following bolus intravenous administration of 5-FU (20 mg/kg) to four other beagle dogs. The commercial preparation of 5-FU for injection (250 mg/5 ml Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) was diluted appropriately with tris(hydroxymethyl)-aminomethane solution, (84.7 mg/ml) and given at a constant rate of 6.2 ml/min with an automatic infusion pump (KN-201, Natsume, Tokyo, Japan). The first blood sample was taken 2.5 min after the start of drug infusion. Then, each saliva sample was periodically collected for 2 min under a liquid paraffin layer by using the method described in the previous paper. Salivation was stimulated with 10% citric acid applied onto the tongue. The blood sample was collected simultaneously, just at the midpoint of the periodical saliva collection intervals.

**Analytical Procedures** — 5-FU concentration in plasma, which was obtained by the centrifugation (3000 rpm, 15 min) of a heparinized blood sample, was determined by an high performance liquid chromatography (HPLC) method which was slightly modified. The modification consisted of a reduction of the sample size by one-half (0.5 ml), the corresponding reductions of the volume in all the extraction steps and the use of acetate buffer (10 mM, pH 4.0) as the mobile phase for separation.

Salivary 5-FU concentration was also determined by the same method described above after a gravimetric determination of the salivary flow rate and measurement of salivary pH. The specific gravity of saliva was assumed to be approximately 1.00.7) Protein levels in plasma and saliva were determined by slightly modifying the method of Lowry et al. using bovine plasma albumin (Fraction V, Armour Pharmaceutical Co., Illinois, U.S.A.) as a standard.

**Data Analysis and Statistical Evaluation** — Following a bolus intravenous administration of 5-FU (20 mg/kg), a log-linear elimination from plasma and saliva was observed in four beagle dogs. Therefore, plasma and saliva concentrations of 5-FU during constant rate infusion were analyzed by the non-linear least-squares microcomputer program MULTI according to the following equation,

$$C = C_{ss} \{1 - \exp(-k_{el} \cdot t)\}$$

(2)

where $C$ is the drug concentration at time $t$. For plasma data, $V_d$ was calculated by Eq. (1). The statistical analysis of the data was performed using the Student’s $t$-test.

**Results and Discussion**

**Plasma and Saliva 5-FU Concentration-time Curve during Constant Rate Intravenous Infusion**

Figure 1 represents 5-FU concentration-time curves for plasma, Pr saliva an MS saliva during constant rate intravenous infusion of 5-FU in three beagle dogs. Immediately after the start of infusion, plasma 5-FU concentration increased very rapidly to achieve a constant level. Salivary 5-FU levels also increased and approached constant values rapidly, but were slower when compared to plasma. Since the time course of plasma 5-FU concentrations following bolus intravenous administration of 20 mg/kg to beagle dogs was described by the one-compartment model, the plasma levels during a constant rate intravenous infusion were analyzed according to Eq. (2). The concentrations of 5-FU in both salivas were fitted to the similar empirical equation, where $C$ is the salivary concentration at time $t$ and $C_{ss}$ is the concentration at steady state. The
weights of 1, 1/C, and 1/C^2 were examined to obtain the best fit. The most probable regression curves derived for plasma and saliva are shown by the solid lines in Fig. 1, and estimated parameter values are represented in Table I.

Plasma half-life calculated was about 5 min. Infusion for a period of six half-lives would attain plasma drug concentrations within 2% of the steady state. Therefore, data obtained after six half-lives, i.e., 30 min after the start of 5-FU infusion, were assumed to be those at the steady state. The estimated steady state 5-FU concentration in plasma was 5.1 µg/ml, which was about half of the expected level. This was due to the fact that both estimated values for k_el and V_d in this study were significantly larger (p < 0.001) than those obtained following bolus intravenous administration in the previous work, which are also represented in Table I.

The correlation between 5-FU concentration in each saliva and plasma was then examined. The relatively scattered but statistically significant correlation was obtained between each saliva and plasma 5-FU concentration as shown in Fig. 2. The slope of the linear regression line for Pr saliva was significantly larger than that for MS saliva (p < 0.05). These findings are consistent with the results following bolus intravenous administration except that the regression lines have no intercepts in the present experiments.

### S/P Ratios and Salivary pH

**Table I.** Pharmacokinetic Parameters for 5-FU during Constant Rate Intravenous Infusion and Following Bolus Intravenous Administration in Beagle Dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>i.v. infusion</th>
<th>Bolus i.v. a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma b)</td>
<td>Pr c)</td>
</tr>
<tr>
<td>C_{ss} (µg/ml)</td>
<td>5.09 ± 0.889 d)</td>
<td>1.97 ± 0.936</td>
</tr>
<tr>
<td>k_el (min⁻¹)</td>
<td>0.140 ± 0.0137</td>
<td>0.0215 ± 0.00659</td>
</tr>
<tr>
<td>V_d (l/kg)</td>
<td>0.428 ± 0.0328</td>
<td>—</td>
</tr>
<tr>
<td>t_{1/2} (min)</td>
<td>4.94 ± 0.484</td>
<td>32.3 ± 9.91</td>
</tr>
<tr>
<td>n</td>
<td>27</td>
<td>24</td>
</tr>
</tbody>
</table>

a) From ref. 5, b) weight = 1/(C_i)^2, c) weight = 1, d) S.D., e) S.E.
Table II. Comparison of Saliva/Plasma 5-FU Concentration Ratios (S/P Ratios) and Salivary pH, Flow Rate, Protein Concentration and Salivary Clearances during Constant Rate Intravenous Infusion and Following Bolus Intravenous Administration in Beagle Dogs

<table>
<thead>
<tr>
<th></th>
<th>i.v. infusion a)</th>
<th>Bolus i.v. b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pr</td>
<td>MS</td>
</tr>
<tr>
<td>S/P ratio</td>
<td>0.192±0.102 c)</td>
<td>0.063±0.038 d)</td>
</tr>
<tr>
<td>n=24</td>
<td>n=22</td>
<td>n=36</td>
</tr>
<tr>
<td>Salivary pH</td>
<td>8.11±0.06</td>
<td>7.84±0.10 d)</td>
</tr>
<tr>
<td>n=19</td>
<td>n=21</td>
<td>n=34</td>
</tr>
<tr>
<td>Salivary flow rate (ml/min/kg)</td>
<td>0.072±0.036</td>
<td>0.163±0.029 d)</td>
</tr>
<tr>
<td>n=24</td>
<td>n=21</td>
<td>n=34</td>
</tr>
<tr>
<td>Protein concn. (mg/ml)</td>
<td>2.58±1.04</td>
<td>1.63±0.54 d)</td>
</tr>
<tr>
<td>n=22</td>
<td>n=21</td>
<td>n=35</td>
</tr>
<tr>
<td>Salivary clearance (ml/min/kg)</td>
<td>0.0162±0.0144</td>
<td>0.0112±0.0074</td>
</tr>
<tr>
<td>n=24</td>
<td>n=21</td>
<td>n=34</td>
</tr>
</tbody>
</table>

a) Plasma 5-FU concentration range was from 1.8 to 6.2 µg/ml. b) Plasma 5-FU concentration range was from 80.6 to 0.2 µg/ml. From ref. 5. c) Mean ± S.D. d) Significantly different from the value for Pr at p < 0.001.

Matin et al. have proposed that the S/P ratio (R) for weak acidic or basic compounds can be predicted from pH-partition hypothesis modified by protein binding in plasma and saliva.¹⁰ For 5-FU, a weak acidic drug, the following equation can be applied:

\[
R = \frac{1 + 10^{(pH - pK_a)}}{1 + 10^{(pH - pK_a)}} \cdot \frac{f_P}{f_S}
\]

(3)

where \( f \) is the free fraction of the total drug concentration; subscripts S and P represent saliva and plasma, respectively. According to this equation, a higher salivary pH will give a larger S/P ratio in this case. Table II summarizes the mean values for the S/P ratios during intravenous infusion and following bolus intravenous administration⁵ together with those for salivary pH, flow rate, protein level and salivary clearance. Both the S/P ratio and salivary pH were significantly higher in Pr than in MS saliva (p < 0.001), and the gland specific differences in this study corresponded with those in the previous paper.⁵ However, each mean S/P ratio tended to be smaller than the ratio in the previous study.⁵

Employing a pH value equal to 7.4 and the reported values for \( pK_a (= 8.1) \),¹¹ \( f_P (= 0.935) \), and \( f_S (= 0.829 \text{ in Pr, } 0.878 \text{ in MS saliva}) \),⁵ the S/P ratios could be calculated by Eq. (3) for the individual pH values of saliva samples observed in this study. The calculated S/P ratios were 2.39 ± 0.28 for Pr saliva (\( n = 19 \)), and 1.39 ± 0.12 for MS saliva (\( n = 21 \)). These values were significantly larger than the corresponding observed ratios, 0.192 for Pr saliva and 0.0634 for MS saliva (\( p < 0.001 \)). No significant correlation was found between the individual observed and predicted S/P ratio of 5-FU in the two salivas (Pr: \( r = -0.023, n = 19, MS: r = -0.147, n = 21 \)).

Therefore, it was suggested that the pH-partition theory or the equation of Matin et al.¹⁰

![Fig. 3. Time Course of S/P Ratio of 5-FU during Constant Rate Intravenous Infusion of 5-FU in Three Beagle Dogs](image-url)

- Pr saliva; ○, MS saliva. Each point represents the mean value ± S.D. a) mean ± range (\( n = 2 \)).
would be only qualitatively applicable in the salivary excretion of 5-FU in beagle dogs.

The relationships were also examined between the S/P ratio of 5-FU and other possible factors, i.e., salivary flow rate and protein concentration in saliva, which could affect the S/P ratios. However, the S/P ratios were not or little influenced by these factors.

Effect of Plasma 5-FU Concentration on the S/P Ratio and Salivary Clearance of 5-FU

Figures 3 and 4 show time courses of the S/P ratio and salivary clearance of 5-FU, respectively, during constant rate intravenous infusion of the drug, where salivary clearance was defined as the salivary drug excretion rate divided by the plasma concentration of the drug, that is, the product of S/P ratio and salivary flow rate.

Apparently, the S/P ratios of 5-FU for both salivas increased with time during the early stage; thereafter, these values approached the steady state levels. The salivary clearance results were similar. On the contrary, the S/P ratios and salivary clearances following bolus intravenous administration were relatively higher shortly after the administration and decreased with time. These findings suggested that the plasma 5-FU concentrations might be a determining factor of the S/P ratio and salivary clearance of 5-FU.

A comparison of coefficients of variation in S/P ratios and salivary clearances of 5-FU between the present infusion and the previous bolus i.v. experiments is presented in Table III. The fluctuations of S/P ratio and salivary clearance of 5-FU were relatively large over the whole sampling period during constant rate infusion. Therefore, it might be difficult to substitute saliva for plasma under this condition. However, these fluctuations were smaller than those following the bolus administration. At the steady state (i.e., after 30 min), the coefficients of variation of S/P ratios in Pr and MS saliva were about one half and one third of the corresponding coefficients after bolus intravenous injection, respectively (Table III). Similarly, for salivary clearance, the coefficients of variation for both salivas were smaller at the steady state than those following bolus intravenous administration of 5-FU. However, the salivary clearance of Pr saliva showed considerably large fluctuations even at the steady state (Table III), which might be due to the relatively large variation of the.

| Table III. Comparison of Coefficient of Variation in S/P Ratio and Salivary Clearance during Constant Rate Intravenous Infusion and Following Bolus Intravenous Administration in Beagle Dogs |
|-----------------|-----------------|-----------------|-----------------|
|                 | S/P ratio       | Salivary clearance |
|                 | Pr              | Pr              | MS              |
| i.v. infusion   |                 |                 |                 |
| Total           | 53.0%           | 60.4%           | 89.3%           | 66.1% |
| n = 24          |                 |                 | n = 24          |      |
| Steady state a) | 28.4%           | 32.0%           | 59.0%           | 40.8% |
| n = 13          |                 |                 | n = 13          |      |
| Bolus i.v. b)   | 64.1%           | 98.0%           | 69.7%           | 102.0% |
| n = 36          |                 |                 | n = 34          |      |

a) 30 min after the start of 5-FU infusion, b) from ref. 5.
Salivary Excretion of 5-FU

Therefore, it was suggested that the S/P ratio and salivary clearance of 5-FU were affected by the plasma 5-FU concentration. The possibility exists that non-linear pharmacokinetics may be involved in the salivary excretion of 5-FU as well as in the disappearance from plasma that was proposed in humans by Collins et al. It was also suggested that the fluctuation of the S/P ratios and salivary clearances of 5-FU were considerably small at the steady state during constant rate intravenous infusion of 5-FU in beagle dogs.

In order to clarify a more detailed mechanism of 5-FU excretion into saliva, additional experiments using wider ranges of plasma 5-FU concentrations and different steady state levels of plasma 5-FU are being conducted. Since such studies are thought to be readily performed with experimental animals other than dogs, rats are being used in this laboratory.

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References