Salivary Excretion of 5-Fluorouracil (5-FU). V. Effect of 5-FU Concentration in Perfusate on the Salivary Excretion of 5-FU in Perfused Rat Mandibular Gland[1)]

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The effect of 5-fluorouracil (5-FU) concentration in the perfusate on the salivary excretion of 5-FU was investigated in the rat mandibular gland perfused with modified Ringer solution containing pilocarpine (10 μM). The saliva to venous-effluent concentration ratio (S/E ratio) of 5-FU increased gradually during the perfusion. The 5-FU concentration in the perfusate ranging from 10 to 200 μg/ml caused elevation in the mean value of S/E ratio. This non-linearity suggested that the present perfusion method would be useful to further investigation for the mechanism of salivary excretion of 5-FU, since the tendency of the non-linearity was similar to that in *in vivo* studies as reported previously. The salivary flow rate declined with time, and the greater mean value of the flow rate was obtained during perfusion with the perfusate containing the lower level of 5-FU. Statistically significant correlation was found between the S/E ratio of 5-FU and salivary flow rate ($p<0.01$). Therefore, in the perfused rat mandibular gland, it was concluded that 5-FU itself had an influence on the salivary excretion of 5-FU *via* decreasing salivary flow rate. On the other hand, the salivary clearance of 5-FU showed no distinct increase and/or decrease not only with time but also with the change of 5-FU concentration in the perfusate. It seems to result from the cancellation of the increased S/E ratio of 5-FU by the decreased salivary flow rate in perfused rat mandibular gland.

Keywords 5-fluorouracil; 5-FU; salivary drug excretion; saliva/venous-effluent drug concentration ratio; salivary clearance; rat; rat saliva; mandibular saliva; perfused rat mandibular gland

Fairly large fluctuations in the saliva/plasma concentration ratios (S/P ratios) and salivary clearances of 5-fluorouracil (5-FU) were observed following bolus intravenous administration (20 mg/kg) of the drug in beagle dogs.[2)] These fluctuations became smaller at the steady state during a constant rate intravenous infusion (0.306 mg/kg/min) in beagle dogs[3)] than those following the bolus administration. To clarify the relation between the S/P ratios and the 5-FU concentration in plasma, increase of the dose to obtain wider ranges of plasma 5-FU concentration was desired. However, the possible acute toxicity of 5-FU made such additional studies with dogs impossible.

The relationship was alternatively investigated employing rats. The S/P ratios and salivary clearances of 5-FU in rats were larger at higher dose, and decreased with the decline of plasma 5-FU concentration following bolus intravenous administration at three dose levels (12.5, 25, and 50 mg/kg), though they showed large fluctuations.[4)] When the change of plasma 5-FU concentration was controlled within relatively narrow ranges in rats by constant rate infusion of the drug, their fluctuations became smaller[5)] than those following the bolus administration in rats, which was similar as in the case with beagle dogs.[2,3)] The larger S/P ratio and salivary clearances were observed at higher dose and higher plasma concentration of 5-FU in rats. It was suggested that non-linear kinetics might be involved, that is, some active process might be concerned in the salivary excretion of 5-FU. It was considered to be necessary to examine the effects of individual factors which might alter salivary excretion of 5-FU in order to elucidate the more detailed mechanism of 5-FU excretion into saliva. However, such studies were difficult to perform in these *in vivo* systems, since, strictly speaking, the plasma 5-FU concentration was not held constant even during constant rate infusion of the drug in rats.

The present study was planned to examine the effect of the 5-FU concentration in perfusate, instead of plasma 5-FU concentration, on salivary excretion of 5-FU itself by using the *in situ* perfusion method in rat mandibular gland, which permits modification of some perfusion conditions. This method was applied for the first time to salivary excretion of the drug, procainamide, in our laboratory,[6)] and was slightly modified in this study. Though similar methods, *i.e.* the partial perfusion method[7)] and the perfusion method with isolation,[8)] in rat mandibular gland have been developed and used dominantly in the field of physiology.

Experimental

Materials 5-FU injection (250 mg/5 ml, Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) and pilocarpine hydrochloride of Japanese Pharmacopeial grade (Hoei Yakko Co., Osaka, Japan) were commercially obtained. 5-Chlorouracil, as an internal standard for the high performance liquid chromatography (HPLC) determination of 5-FU, was kindly supplied by Otsuka Pharmaceutical Factory, Inc., Naruto, Japan. *N*-(2-Hydroxyethyl)-piperazine-*N'*-2-ethanesulfonic acid (HEPES) was purchased from Nakalai Tesque, Inc., Kyoto, Japan. All other reagents and solvents were of analytical grade.

Animals Male Wistar rats (weighing 340—440 g, 12 to 17 weeks-old) purchased from Shizuoka Laboratory Animals Center (Hamamatsu, Japan) were used with feeding *ad lib.* Each rat was anesthetized with pentobarbital sodium (50 mg/kg) intraperitoneally. Body temperature was thermostatically kept between 37 and 38 °C by using heated dissection pads placed under the supine rats.

Perfusion of Mandibular Gland and Collection of Venous Effluent and Saliva Samples The procedures employed in the present study were essentially the same as those described previously by Iwamoto *et al.*[6)] Briefly, the duct orifice of mandibular gland was cannulated with a polyethylene tubing (PE-10, Clay Adams, Parsippany, New Jersey, U.S.A.) to collect saliva samples, the gland was dissected, and the sublingual gland was ligated. The arterial supply to the gland was perfused through a cannula (PE-10) inserted in the external mandibular artery to within a few mm of the glandular branch. The venous effluent draining the gland was collected through a cannula (PE-50) inserted in the glandular tributary. All other branches were ligated to perfuse only the gland.

The perfusion was carried out with a modified Ringer solution.[8j)] The composition of the perfusate was (in mM): Na^+ 146.0, K^+ 4.3, Ca^+ 1.0, Mg^{2+} 1.0, Cl^- 146.3, SO_4^{2-} 1.0, PO_4^{3-} 1.0, and glucose 5.0. The solution was buffered at pH 7.4 with HEPES (10 mM), gassed with 100% O_2, and kept about 37 °C.[8j)] This modified Ringer solution was perfused arterially with the aid of a peristaltic pump (Minipuls 2, Gilson Co., Middleton,

Wisconsin, U.S.A.) at a rate of 1.5 ml/min without recirculation. The concentration of 5-FU was prepared at 0 (control), 10, 50, 100, or 200 μg/ml. Each perfusion medium contained pilocarpine hydrochloride (10 μM) as a stimulant for salivation.[6]

After a washout time of 30 s for blood in the mandibular gland, both serial venous effluent and saliva samples, pooled for 10 min, were collected over 90 min in preweighed tubes and microsample tubes, respectively. For measurement of salivary pH, saliva samples were led to the bottom of the tube containing a liquid paraffin (about 100 μl) through polyethylene tubing.

Analytical Procedures Salivary pH was determined by the same methods described previously.[4] Salivary flow rate was determined gravimetrically assuming the specific gravity of saliva to be equal to 1.0.[4] Salivary flow rate was expressed as saliva volume per min per the body weight or saliva volume per min per non-perfused gland wet weight. For the purpose of the expression, both the perfused and non-perfused mandibular glands were dissected and weighed at the end of each experiment. Both venous effluent and saliva samples (50 μl) were analyzed for 5-FU by the same HPLC method as described previously.[4] The statistical evaluation of data was performed by using the Student's *t*-test.

Results and Discussion

Effect of the Perfusate 5-FU Level on the Saliva/Venous-Effluent Concentration Ratio (S/E Ratio) of 5-FU in the Perfused Rat Mandibular Gland Figure 1 shows the time course of the S/E ratio for 5-FU in rat mandibular gland perfused and stimulated with modified Ringer solution containing 10 μM pilocarpine. In all cases, the S/E ratio of 5-FU increased gradually with time and reached a few times as large value as the initial one, though the 5-FU concentrations in perfusate, accordingly those in venous effluent, remained unchanged. Such increasing tendency has not been noticed with the salivary excretion of procainamide studied by a similar perfusion method,[6] and might suggest the impairment of the mandibular gland by 5-FU as discussed later.

As shown in Fig. 2, the increase of 5-FU concentration in perfusate resulted in raising the S/E ratio of 5-FU with regard to the pooled data for all sampling points; all the S/E ratios differed significantly at $p<0.001$ from others except for 50 *vs.* 100 μg/ml of 5-FU concentration in the perfusate. This tendency is consistent with the previously reported results of *in vivo* studies in rats, where the higher plasma concentration of 5-FU caused the larger S/P ratio.[4,5] Since the non-linearity of salivary excretion of 5-FU was retained in this *in situ* perfusion method, this technique is considered useful to examine the effects of

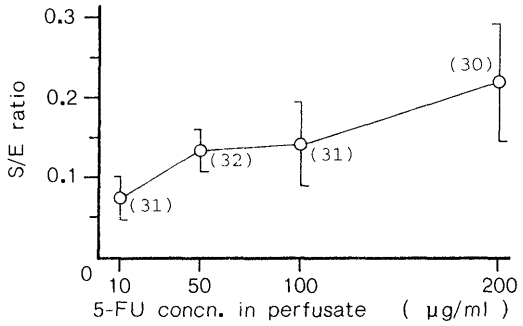

Fig. 2. Effect of the Perfusate 5-FU Level on Its S/E Ratio in the Perfused Rat Mandibular Gland

Each point represents the mean±S.D. of four rats. The figure in parentheses represents number of observed data points. All S/E ratios differed significantly at $p<0.001$ from others except for 50 *vs.* 100 μg/ml of 5-FU concn. in perfusate.

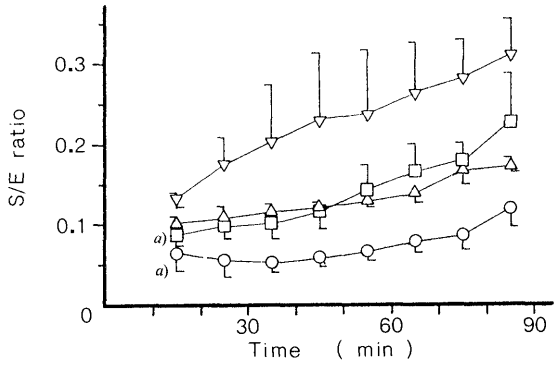

Fig. 1. Saliva/Venous-Effluent Concentration Ratio (S/E Ratio) of 5-FU in the Perfused Rat Mandibular Gland

5-FU concn. in perfusate: 10 (○), 50 (△), 100 (□), or 200 (▽) μg/ml. Each point represents the mean±S.D. of four rats. *a*) The mean±S.D. of three rats.

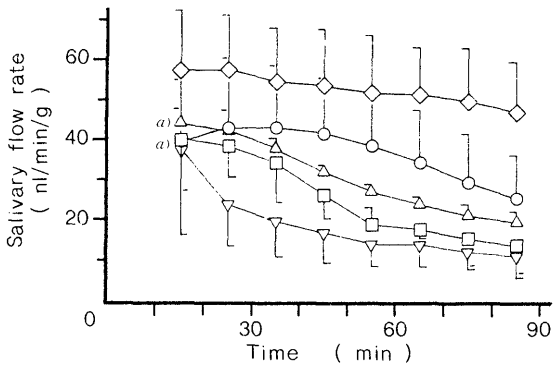

Fig. 3. Salivary Flow Rate in the Perfused Rat Mandibular Gland

5-FU concn. in perfusate: 0 (control, ◇), 10 (○), 50 (△), 100 (□), or 200 (▽) μg/ml. Each point represents the mean±S.D. of three rats for control or four rats for the others. *a*) The mean±S.D. of three rats.

TABLE I. Saliva/Venous-Effluent Concentration Ratio (S/E Ratio) of 5-FU, Salivary Flow Rate, Salivary Clearance of 5-FU, and Salivary pH in the Perfused Rat Mandibular Glands

5-FU concn. in perfusate (μg/ml)		0 (Control) (24,[a] 3[b])	10 (31, 4)	50 (32, 4)	100 (31, 4)	200 (30, 4)
S/E ratio			0.0734±0.0274	0.134±0.0264	0.141±0.0534	0.218±0.0735
Flow rate[c]	(nl/min/g)	52.7±12.2	37.1±15.2[d]	31.6±9.86[e]	27.0±10.7[e]	19.7±10.2[e]
	(nl/min/mg)	89.1±37.7	66.7±34.8	68.0±27.2	34.4±15.4[e]	31.7±18.3[e]
Clearance[c]	(nl/min/g)		2.59±1.13	4.02±0.722	3.07±0.801	3.59±1.17
	(nl/min/mg)		4.69±2.54	8.56±2.07	4.21±1.06	6.12±2.88
pH		7.31±0.13	7.46±0.21[e]	7.36±0.08[d]	7.32±0.09[f]	7.38±0.09[e,f]

Each value represents the mean±S.D. *a*) Number of the data points. *b*) Number of the rats. *c*) Salivary flow rate and clearance were expressed as volume per min per body weight (g) or per gland wet weight (mg) of non-perfused side. *d*) Significantly different from the control value at $p<0.01$. *e*) Significantly different from the control value at $p<0.001$. *f*) $n=28$.

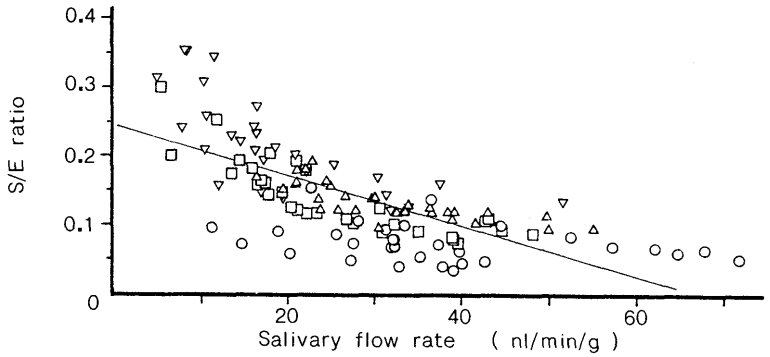

Fig. 4. Correlation between S/E Ratio of 5-FU and Salivary Flow Rate in the Perfused Rat Mandibular Gland

5-FU concn. in perfusate: 10 (○), 50 (△), 100 (□), or 200 (▽) μg/ml. Regression line: $Y=-0.00361X+0.243$ ($r=0.700$, $n=124$ from 12 rats, $p<0.01$), where Y is S/E ratio and X is salivary flow rate.

individual factors which might alter salivary excretion of 5-FU in rats.

The effect of salivary pH on S/E ratio of 5-FU was examined. There was no significant relationship between the observed S/E ratio of 5-FU and salivary pH. No statistically significant relationship was also found between the observed S/E ratio and the S/E ratio calculated employing its salivary pH according to the equation of Matin *et al.*[9] Therefore, in this system, salivary pH had little effect on the S/E ratio of 5-FU.

Salivary Flow Rate in the Perfused Rat Mandibular Gland The time course of salivary flow rate is illustrated in Fig. 3. At the highest 5-FU concentration in the perfusate (200 μg/ml), the salivary flow rate declined with time remarkably to a plateau value of *ca.* 12 nl/min/g after 50 min. At lower 5-FU concentrations in the perfusate, declines of the salivary flow rate were delayed, and their extents were smaller. As the control experiment, mandibular glands were perfused with the perfusate without 5-FU. In the control, also shown in Fig. 3, the salivary flow rate was maintained at higher level than that for any other perfusion experiment with 5-FU, although it declined slightly with time.

The comparison of the mean value for the salivary flow rate among each 5-FU concentration in the perfusate has revealed that increase of 5-FU concentration in the perfusate resulted in decrease of the salivary flow rate. This is indicated in Table I with regard to the pooled data of all sampling points which summarizes the S/E ratio of 5-FU, the salivary flow rate and the salivary clearance of 5-FU as well as the salivary pH.

Furthermore the relationship between the S/E ratio of 5-FU and salivary flow rate was examined in Fig. 4. A statistically significant correlation was found between the S/E ratio of 5-FU and salivary flow rate ($p<0.01$). These findings suggested that 5-FU might affect the salivary flow rate.

Kim and Han[10] have reported regarding mice that body weights and weights of three major salivary glands increased following three daily intramuscular injections of 25 mg/kg of 5-FU. Since injection of pilocarpine prior to sacrifice abolished the increase in glandular weights of salivary glands, they thought this increase in the gland weights was due to the inability of salivary glands to release synthesized secretory products. This report supports the results obtained in the present study, though experimental conditions were not necessarily the same. It was suggested

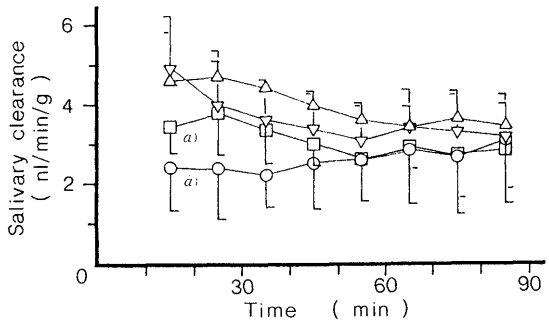

Fig. 5. Salivary Clearance of 5-FU in the Perfused Rat Mandibular Gland

5-FU concn. in perfusate: 10 (○), 50 (△), 100 (□), or 200 (▽) μg/ml. Each point represents the mean±S.D. of four rats. *a*) The mean±S.D. of three rats.

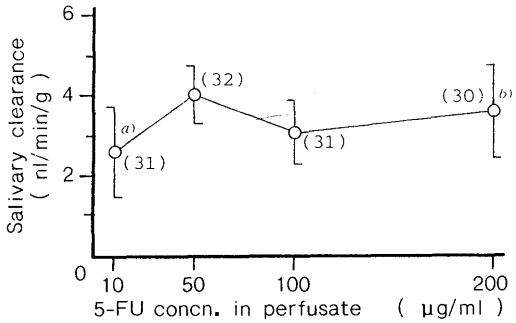

Fig. 6. Effect of the Perfusate 5-FU Level on Its Salivary Clearance in the Perfused Rat Mandibular Gland

Each point represents the mean±S.D. of four rats. The figure in parentheses represents number of observed data points. *a*) Significantly different from 50 and 200 μg/ml of 5-FU concentration in perfusate at $p<0.001$ and $p<0.01$, respectively. *b*) Significantly different from 50 and 200 μg/ml of 5-FU concentration in perfusate at $p<0.001$ and $p<0.05$, respectively.

that 5-FU might diminish fluid secretion by perfused rat mandibular gland, and that owing to the decrement of the fluid secretion the S/E ratio of 5-FU might increase with the 5-FU concentration in the perfusate.

In previous papers,[4,5] significant correlations have not been observed between the S/P ratios and salivary flow rates both following bolus intravenous administration of 5-FU and during constant rate infusion of 5-FU in rats. It may be interpreted that considerably large inter- and/or intra-individual variations in salivary flow rate as well as those in plasma 5-FU concentration, masked the effects of salivary flow rate on salivary excretion of 5-FU, or the S/P

ratios in these *in vivo* studies.

Effect of the Perfusate 5-FU Level on the Salivary Clearance of 5-FU in the Perfused Rat Mandibular Gland Figures 5 and 6 show the time course of the salivary clearance of 5-FU and the effect of 5-FU level in the perfusate on the salivary clearance of 5-FU in a perfused rat mandibular gland, respectively. The salivary clearance was calculated as the salivary drug excretion rate divided by the drug concentration in plasma or venous-effluent by the definition.[11] That is, it was obtained as the product of S/E ratio and salivary flow rate in this study. There was no distinct increase and/or decrease with time in the salivary clearance of 5-FU. The mean value of the salivary clearance at 10 and 100 μg/ml of 5-FU concentration in the perfusate were significantly different ($p < 0.05$—0.001) from those at 50 and 200 μg/ml of 5-FU concentration in the perfusate. However, the rank order of the salivary clearance of 5-FU did not agree with that of the perfusate 5-FU concentration (Table I). It may be appropriately concluded that the 5-FU concentration in perfusate ranging from 10 to 200 μg/ml had little effect on the salivary clearance of 5-FU in perfused rat mandibular gland. This phenomenon seems to result from the cancellation of the increased S/E ratio of 5-FU by the decreased salivary flow rate.

In conclusion, the present *in situ* perfusion method proved to be a useful technique to investigate the salivary excretion mechanism of 5-FU. Some further studies employing the method have been continued in our laboratory for elucidation of the specific transport mechanism involved in salivary excretion of 5-FU.

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References and Notes

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