[Chem. Pharm. Bull. 36(11)4547—4553(1988)]

Salivary Excretion of 5-Fluorouracil (5-FU). III. Non-linear Kinetics of Salivary Excretion of 5-FU Following Bolus Intravenous Administration in Rats[1)]

YAYOI HAYASHI,* JUN WATANABE, SHOJI OZEKI, and KIKUO IWAMOTO[2)]

Department of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1, Tanabe-dori, Mizuoho-ku, Nagoya 467, Japan

(Received April 11, 1988)

Salivary excretion profiles of 5-fluorouracil (5-FU) were investigated following bolus intravenous administration at three dose levels (12.5, 25, and 50 mg/kg) in rats. Mandibular (M) and parotid (Pr) saliva samples were periodically collected separately *via* cannulas inserted into the ducts by stimulating salivation with pilocarpine infused intravenously. Simultaneously, salivary pH, flow rate, and protein concentration were determined.

(1) Plasma and saliva 5-FU concentration decreased bi-exponentially with time. A relatively scattered relationship but a statistically significant correlation was found between each of the saliva concentrations and plasma 5-FU concentration ($p<0.01$). (2) The saliva/plasma concentration ratios (S/P ratios) and salivary pH were higher in M than Pr saliva, in contrast to the results in beagle dogs. (3) The S/P ratio and salivary clearance of 5-FU were larger at higher dose, and decreased with the decline of plasma 5-FU concentration, though they showed large fluctuations. It was, therefore, suggested that non-linear kinetics might be involved in the salivary excretion of 5-FU in rats. (4) The contribution of total salivary clearance to total body clearance of 5-FU was found to be insignificant, *i.e.* less than 0.1% even at the highest dose.

Keywords——5-fluorouracil; salivary drug excretion; salivary drug concentration; saliva/plasma drug concentration ratio; salivary clearance; non-linear kinetics; rat; rat saliva; mandibular saliva; parotid saliva

From the point of view of therapeutic drug monitoring, the salivary excretion of various drugs has been rather well documented in man.[3)] However, there have been as yet few reports investigating the mechanisms of salivary excretion of drugs in detail.

In previous papers, to elucidate the salivary excretion mechanism of drugs, we established experimental methods for the periodical collection of parotid and mandibular-sublingual saliva samples separately in dogs by producing permanent fistulae,[4a)] and evaluated the effects of salivary pH, flow rate, or protein binding on the saliva/plasma drug concentration ratio (S/P ratio) and/or the salivary clearance of various drugs.[1,4)] For the salivary excretion of 5-fluorouracil (5-FU), which is thought to require the routine monitoring of the plasma levels to enhance the effectiveness and to minimize the toxic side effects, the S/P ratios and the salivary clearances showed large fluctuations following bolus intravenous administration (20 mg/kg) of the drug in beagle dogs.[4d)] During a constant rate intravenous infusion,[4e)] the fluctuations became smaller at the steady state than those following bolus administration. It was suggested that the S/P ratio and salivary clearance of 5-FU were affected by the plasma drug concentration in beagle dogs. In order to elucidate in more detail the mechanism of 5-FU excretion into saliva, additional investigations using wider ranges of

plasma 5-FU concentrations are needed. However, such studies are difficult to perform with dogs.

On the other hand, in the field of physiology, especially oral physiology, many studies have been carried out in rats on the salivary secretion or excretion of water and/or some endogenous electrolytes such as Na^+ or K^+. There are also several reports dealing with drug excretion into saliva in rats.[5] In one of them, it was reported that 5-FU was excreted in detectable amounts into parotid saliva following bolus intravenous administration to rats.[5f] However, detailed pharmacokinetic evaluation of the salivary drug data was not feasible bacause of the limited number of saliva samples which were pooled for relatively long periods. Most other studies performed with rats have similar limitations. Therefore, we recently established experimental methods, comparable to those in beagle dogs[1,4] to examine the salivary excretion kinetics of drugs in rats,[6] or rabbits.[7]

In the present study using rats as model animals, the salivary excretion kinetics of 5-FU was examined following bolus intravenous administration with a relatively wide range of dose levels and the results were compared with our previous results in beagle dogs.[4d]

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. All other reagents and solvents were of analytical grade. A constant-rate infusion pump (KN-201, Natsume Seisakusho Co., Tokyo, Japan) for promoting salivation by pilocarpine infusion, and a micro glass electrode (SE-1700GC, Fujikagaku Keisoku, Mitaka, Japan) for determination of salivary pH were used.

Animals——Male Wistar rats (weighing 400—500 g, 13- to 17-week-old) utilized in this study were purchased from Shizuoka Laboratory Animals Center (Hamamatsu, Japan). Rats were anesthetized with pentobarbital (50 mg/kg) intraperitoneally after overnight fasting for over 15 h. Body temperature was thermostatically kept between 37 and 38°C by using heated dissection pads placed under the supine rats.

Drug Administration and Collection of Blood and Saliva Samples——After tracheotomy and catheterization, cannulae were made by the method described by Watanabe *et al.*[6] The femoral vein was cannulated with a heparinized polyethylene tubing (PE-50, Clay Adams, Parsippany, New Jersey, U.S.A.) for infusion of pilocarpine hydrochloride at constant rate of 5.0 mg (free base)/kg/h to stimulate salivation. The jugular vein was also cannulated with heparinized silicone polymer tubing (i.d. 1.0 mm; o.d. 1.5 mm, Dow Corning, Tokyo, Japan) for administration of 5-FU and for collection of blood samples. Beveled polyethylene tubing (PE-10, Clay Adams) was inserted into each one of the mandibular (M) and parotid (Pr) duct orifices in the buccal cavity to collect saliva samples separately.

Following constant rate infusion of pilocarpine for 2 h, when salivation had reached the steady state, 5-FU injection appropriately diluted with tris(hydroxymethyl)aminomethane solution (84.7 mg/ml) was administered intravenously as a bolus dose of 12.5, 25, or 50 mg/kg to rats. Saliva samples were collected at various times for 10 to 20 min under a liquid paraffin layer (about 0.1 ml) by a method similar to that described previously.[4b] Blood samples were taken 2 and 6 min after the administration and at the midpoint of the periodical saliva collection intervals and were centrifuged to obtain plasma.

Analytical Procedures——Salivary flow rate was determined gravimetrically assuming the specific gravity of saliva to be 1.0,[4b] and was expressed as saliva volume per min per body weight. Salivary pH was measured immediately after aspiration of the liquid paraffin, and was referred to the value at 3 min after immersing the electrode.

5-FU concentrations in plasma and saliva were determined by the HPLC method as described previously,[4e] modified by a reduction of the sample size to 50 μl and corresponding reduction of the volume in all of the extraction steps. Saliva samples of less than 50 μl were appropriately diluted to produce this sample size by adding blank saliva.

Protein levels in plasma and saliva were determined by a slight modification of the method of Lowry *et al.*[8] using bovine plasma albumin (Fraction V, Amour Pharmaceutical Co., purchased from Sanko Pure Chemicals Co., Tokyo, Japan) as a standard.

Data Analysis and Statistical Evaluation——Plasma and saliva concentrations of 5-FU were analyzed according to the least-squares regression program for a two-compartment model. The statistical analysis of data was performed by using Student's *t*-test.

Results and Discussion

Plasma and Saliva Concentrations of 5-FU Following Bolus Intravenous Administration

Figure 1 shows 5-FU concentration–time curves for plasma, M saliva and Pr saliva following bolus intravenous administration of the drug to rats at low (12.5 mg/kg), medium (25 mg/kg) and high (50 mg/kg) doses. The drug concentrations in plasma and saliva decreased biexponentially with time. Although Pr saliva concentration data at low dose could not be analyzed, probably because of the limited number of data points in the distribution phase, the other concentration data could be analyzed in terms of bi-exponential equations as indicated by the computer-fitted solid lines in Fig. 1. The observed concentrations in M saliva are scattered around the best-fit curves due to the relatively large variations. Estimated pharmacokinetic parameters for 5-FU in plasma are summarized in Table I. All values for the total body clearance of 5-FU in rat at the three dose levels were slightly smaller than that in beagle dogs (0.0306 ± 0.005 ml/min/g) following bolus intravenous administration of 20 mg/kg.[4d]

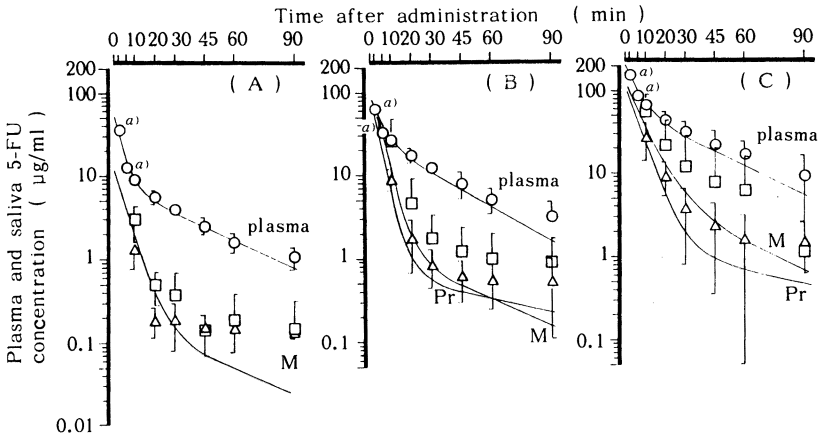

Fig. 1. Plasma and Saliva 5-FU Concentrations Following Bolus Intravenous Administration of Low (A), Medium (B), and High (C) Doses to Rats

Dose: (A) 12.5 mg/kg, (B) 25 mg/kg, (C) 50 mg/kg. ○, plasma; □, mandibular (M) saliva; △, parotid (Pr) saliva. Each point represents the mean ± S.D. from three to six rats [*a*) mean ± range ($n=2$)]. The solid lines show the most probable computer-fitted curves.

TABLE I. Pharmacokinetic Parameters for 5-FU in Plasma Following Bolus Intravenous Administration of 5-FU to Rats (Value for Parameter ± S.E.[a])

Parameter		Dose (mg/kg)	Low 12.5	Medium 25	High 50
A	(μg/ml)		44.2 ± 13.1	51.0 ± 26.6[b]	129 ± 50.6[b,c]
B	(μg/ml)		7.77 ± 0.91	27.9 ± 4.9[b]	57.3 ± 10.7[b]
α	(min^{-1})		0.280 ± 0.058	0.227 ± 0.113[b]	0.178 ± 0.071[b,c]
β	(min^{-1})		0.0257 ± 0.0212	0.0331 ± 0.0031[b]	0.0255 ± 0.0030[d]
$t_{(1/2)\beta}$	(min)		26.9 ± 3.2	22.3 ± 2.2[b]	27.2 ± 3.2[c]
Vd_{ss}	(ml/g)		0.728 ± 0.252	0.593 ± 0.365	0.523 ± 0.264[b]
CL_{tot}[e]	(ml/min/g)		0.0269 ± 0.0022	0.0223 ± 0.0147	0.0169 ± 0.0091[b]
n			40	39	40

All data weights (i) = $1/Ci^2$. *a*) W. E. Deming, "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946. *b*) Significantly different from the low dose at $p<0.001$. *c*) Significantly different from the medium dose at $p<0.05$. *d*) Significantly different from the medium dose at $p<0.001$. *e*) Calculated as $V_1 \times k_{10}$.

A relatively scattered relationship, but statistically significant correlation, was found between plasma concentration and each of the saliva 5-FU concentrations at every dose. For the combined data at the three dose levels, there was a rather good correlation between the concentrations in plasma (X) and in each saliva (Y). The regression lines were $Y=0.788X-4.46$ ($r=0.806$, $n=102$, $p<0.01$) for M saliva and $Y=0.342X-2.18$ ($r=0.842$, $n=92$, $p<0.01$) for Pr saliva. The slope of the regression line for M saliva was significantly larger than that for Pr saliva ($p<0.001$). This result indicates the existence of a gland-specific defference in salivary excretion of 5-FU in rats.

Comparison of the *S/P* Ratio and Salivary pH

Table II summarizes the mean values for the S/P ratios of 5-FU and salivary pH following bolus intravenous administration of 5-FU to rats, together with the data in beagle dogs.[4d] In rats, the observed S/P ratios were significantly larger in M than Pr saliva ($p<0.001$) and the salivary pH values in M saliva were also significantly higher than those in Pr saliva. These findings were in contrast to the results in beagle dogs,[4d] that is, the observed S/P ratios and salivary pH of dogs were significantly higher ($p<0.001$) in Pr than in mandibular-sublingual saliva which was collected instead of M saliva alone, though salivation was gustatorily stimulated with 10% citric acid in beagle dogs.

The predictive S/P ratios were calculated according to the equation of Matin *et al.*,[9] which was based on the pH-partition hypothesis and modified to take account of protein binding, and are also listed in Table II. In the calculation, the pH values measured in individual saliva samples (pH_S) and the following values were employed: $pK_a=8.1$,[10] the pH value of plasma (pH_P)$=7.4$, the free fraction of the total drug concentration in plasma (f_P)$=0.9$,[5f] and that in saliva (f_S)$=1.0$ (assumed). In rats, a higher salivary pH in M saliva than that in Pr saliva gave a larger calculated S/P ratio in M saliva than that in Pr saliva, whereas the reverse was true in beagle dogs.[4d] Poor but significant correlations were found between the individual observed and predicted S/P ratios of 5-FU in both salivas (M saliva: $r=0.407$, $n=100$, $p<0.01$; Pr saliva: $r=0.218$, $p<0.01$, $n=92$). However, the calculated values were much larger than the corresponding observed ratios in both salivas ($p<0.001$) as

TABLE II. Comparison of Observed and Calculated S/P Ratios of 5-FU, Salivary pH, Flow Rate, and Protein Concentration Following Bolus Intravenous Administration of 5-FU to Rats and Beagle Dogs (Mean ± S.D.)

	Rats[a]		Beagle dogs[b]	
	M	Pr	MS[c]	Pr
S/P ratio				
Observed	0.291 ± 0.347[d] (102[e])	0.137 ± 0.115 (92[e])	0.200 ± 0.196[d] (31[e])	0.473 ± 0.303 (36[e])
Calculated[f]	2.21 ± 0.522[d] (100[e])	1.19 ± 0.152 (92[e])	1.59 ± 0.130[d] (31[e])	2.05 ± 0.146 (34[e])
Salivary pH	8.36 ± 0.15[d] (100[e])	7.84 ± 0.14 (92[e])	7.98 ± 0.07[d] (31[e])	8.10 ± 0.06 (34[e])
Flow rate (nl/min/g)[g]	13.5 ± 9.72 (102[e])	16.4 ± 6.84 (92[e])	99.7 ± 19.9 (31[e])	99.3 ± 25.6 (34[e])
Protein concn. (mg/ml)	2.42 ± 1.11[d] (82[e])	3.33 ± 1.48 (81[e])	1.71 ± 0.86 (32[e])	1.93 ± 1.48 (35[e])

a) Salivation was stimulated with pilocarpine. *b*) From Ref. 4*d*. Dose: 20 mg/kg. Salivation was stimulated with 10% citric acid. *c*) Mandibular-sublingual saliva. *d*) Significantly different from the corresponding value for Pr saliva at $p<0.001$. *e*) Number of data points. *f*) Calculated by using Matin's equation: $R=[1+10^{(pH_S-pK_a)}]/[1+10^{(pH_P-pK_a)}]\times f_P/f_S$. *g*) Expressed as saliva volume per min per body weight (1 nl/min/g × 1 μl/min/kg).

shown in Table II. Therefore, the present gland type differences between M and Pr in S/P ratios of 5-FU in rats are considered to result from gland-specific differences in the salivary pH. In addition, the species differences in the S/P ratios between rats and beagle dogs may be attributed to species-dependent differences in the salivary pH itself.

The mean values for salivary flow rate and protein concentration in saliva are also included in Table II. No distinct relationships were observed between the S/P ratios of 5-FU and either of these two factors. Therefore, neither salivary flow rates nor protein concentrations could be considered responsible for the gland and species differences and the fluctuation of the S/P ratios for 5-FU in rats and beagle dogs.

Effects of Dose and Plasma 5-FU Concentration on S/P Ratio and Salivary Clearance of 5-FU

The coefficients of variation (C.V.) in the observed S/P ratios of 5-FU in rats (Table II) were 119% and 83.9% for M and Pr saliva, respectively. Values of salivary clearance, which has been defined as the salivary drug excretion rate divided by the plasma concentration of the drug,[4c,11] and was calculated for all data, were 2.93 ± 3.18 ($n=102$, C.V. = 109%) and 2.15 ± 2.01 nl/min/g ($n=92$, C.V. = 93.5%) for M and Pr saliva, respectively. Both the S/P ratios and salivary clearances of 5-FU showed large fluctuations. These fluctuations were not less than those in beagle dogs (S/P ratio: MS, 98.0%; Pr, 64.1%. Salivary clearance: MS, 102%; Pr, 69.7%) following bolus intravenous administration,[4d] and the range of plasma 5-FU concentrations (80.6—0.2 μg/ml) was comparable to that in rats.

In Table III, the mean S/P ratios of 5-FU are compared among the three dose levels. They were larger at higher dose, though large fluctuations were seen in both salivas at each

Table III. Comparison of S/P Ratios of 5-FU Following Bolus Intravenous Administration of 5-FU at Three Dose Levels to Rats (Mean ± S.D.)

Dose (mg/kg)	Low 12.5	Medium 25	High 50
M	0.162 ± 0.212	$0.325 \pm 0.412^{a)}$	$0.403 \pm 0.356^{b)}$
	$36^{c)}$	$34^{c)}$	$32^{c)}$
Pr	0.088 ± 0.059	$0.147 \pm 0.110^{d)}$	$0.170 \pm 0.141^{b)}$
	$29^{c)}$	$29^{c)}$	$34^{c)}$

a) Significantly different from the low dose at $p<0.001$. *b*) Significantly different from the low dose at $p<0.01$. *c*) Number of data points. *d*) Significantly different from the low dose at $p<0.05$.

Table IV. Salivary Clearance and Total Body Clearance Following Bolus Intravenous Administration of 5-FU at Three Dose Levels to Rats

	Dose (mg/kg)	Low 12.5	Medium 25	High 50
Salivary clearance[a)] (nl/min/g)	M	2.38 ± 2.24	3.13 ± 3.55	3.36 ± 3.63
		$36^{b)}$	$34^{b)}$	$32^{b)}$
	Pr	1.59 ± 0.92	2.16 ± 2.08	$2.63 \pm 2.50^{c)}$
		$29^{b)}$	$29^{b)}$	$34^{b)}$
	Total[d)]	8.15 ± 5.80	10.7 ± 11.1	12.2 ± 11.6
		$29^{b)}$	$29^{b)}$	$30^{b)}$
Total body clearance[e)] (μl/min/g)		26.9 ± 2.22	22.3 ± 14.7	$16.9 \pm 9.11^{f)}$
		$40^{b)}$	$39^{b)}$	$40^{b)}$

a) Mean ± S.D. *b*) Number of data points. *c*) Significantly different from the low dose at $p<0.05$. *d*) Calculated by doubling the sum of salivary clearance for M and Pr saliva. *e*) These values are the same as CL_{tot} in Table I. *f*) Significantly different from the low dose at $p<0.001$.

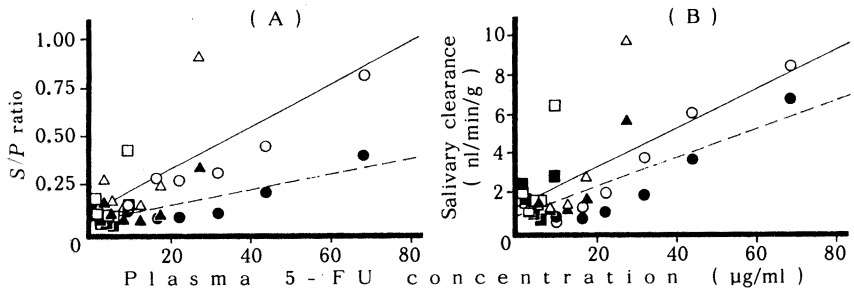

Fig. 2. Correlation between S/P Ratio (A) or Salivary Clearance (B) and Plasma Concentration of 5-FU Following Bolus Intravenous Administration of 5-FU to Rats

○— △— □—, M saliva; ●--- ▲--- ■---, Pr saliva. Dose: 12.5 mg/kg (□, ■), 25 mg/kg (△, ▲), or 50 mg/kg (○, ●). Each point represents the mean value for the same sampling time obtained from three to six rats.

The regression lines calculated with the original data were as follows: (A) for M saliva, $Y=0.110X+0.113$ ($r=0.576$, $n=102$), for Pr saliva, $Y=0.00390X+0.0697$ ($r=0.637$, $n=92$); (B) for M saliva, $Y=0.101X+1.30$ ($r=0.576$, $n=102$), for Pr saliva, $Y=0.0748X+0.863$ ($r=0.697$, $n=92$). All correlations were significant at $p<0.01$.

dose level. Salivary clearances of 5-FU showed a similar tendency, being slightly larger at higher dose, as shown in Table IV. These results suggested that salivary excretion of 5-FU was influenced by the dose.

Figure 2 shows the relationship between S/P ratio or salivary clearance of 5-FU and the plasma concentration. Panels A and B illustrate the scatter of the S/P ratio and the salivary clearance, respectively. Both S/P ratio and salivary clearance of 5-FU had significant correlations with plasma 5-FU concentration for each saliva ($p<0.01$), showing that the salivary excretion of 5-FU is dependent on the plasma concentration. Therefore, it was suggested that non-linear kinetics might be involved in the salivary excretion of 5-FU in rats.

Comparison of Salivary Clearance with Total Body Clearance

When salivation can be continuously stimulated, salivary excretion of cetain drugs or chemicals may be considered as one of the elimination or removal routes in some emergency situations, *e.g.* drug intoxication.[4c] Total salivary clearance of 5-FU was compared with total body clearance following bolus intravenous administration of 5-FU at three dose levels in rats (Table IV). Double the sum of M and Pr salivary clearances obtained at the same sampling time was taken as total salivary clearance, since both saliva samples were collected only from one of each pair of salivary glands. The value even at the highest dose (12.2 nl/min/g) corresponded to only 0.07% of the total body clearance (16.6×10^3 nl/min/g). Therefore, it was indicated that even under the condition of stimulated salivation, salivary excretion would not play an important role in the overall elimination of 5-FU from the body in rats, as in beagle dogs.[4d]

In conclusion, the gland type differences in the S/P ratio of 5-FU in rats are considered to result from gland-specific differences in salivary pH, and the species differences between rats and beagle dogs may be attributed to be species-dependent differences in their salivary pH. Similarly to the results in beagle dogs, both S/P ratios and salivary clearances of 5-FU were larger at higher dose and decreased with the decline of plasma 5-FU concentrations following bolus intravenous administration in rats. These findings suggest that non-linear kinetics may be involved in the salivary excretion of 5-FU in rats. The contribution of salivary excretion to the overall elimination of 5-FU from the body appeared negligible even under the condition of stimulated salivation in rats.

Additional studies on 5-FU excretion into saliva are being conducted in this laboratory

by infusing 5-FU intravenously at a few different constant rates into rats to reduce the fluctuation of data.

References and Notes

1) This paper constitutes Part X of the series entitled "Salivary Excretion of Drugs." Part IX: J. Watanabe, H. Murata, J. Higuchi, Y. Hayashi, K. Iwamoto, H. Yuasa, and S. Ozeki, *Chem. Pharm. Bull.*, **36**, 3105 (1988).
2) Present address: *Department of Pharmacy, Shimane Medical University Hospital, 89–1, Enya-cho, Izumo 693, Japan.*
3) M. Danhof and D. D. Breimer, *Clin. Pharmacokinet.*, **3**, 39 (1978); J. C. Mucklow, M. R. Bending, G. C. Kahn, and C. T. Dollery, *Clin. Pharmacol. Ther.*, **24**, 563 (1978); J. C. Mucklow, *Ther. Drug Monit.*, **4**, 229 (1982).
4) *a*) J. Watanabe, Y. Urasaki, Y. Nakase, H. Ueda, K. Iwamoto, and S. Ozeki, *J. Pharmacobio-Dyn.*, **4**, 336 (1978); *b*) J. Watanabe, Y. Nakase, Y. Urasaki, Y. Hayashi, K. Iwamoto, and S. Ozeki, *ibid.*, **4**, 968 (1981); *c*) J. Watanabe, S. Mizuno, N. Masuda, Y. Hayashi, K. Iwamoto, J. Hirate, and S. Ozeki, *ibid.*, **7**, 294 (1984);*d*) J. Watanabe, Y. Hayashi, K. Iwamoto, and S. Ozeki, *Chem. Pharm. Bull.*, **33**, 1187 (1985); *e*) Y. Hayashi, J. Watanabe, K. Iwamoto, and S. Ozeki, *J. Pharmacobio-Dyn.*, **11**, 438 (1988).
5) *a*) A. J. Piraino, G. J. DiGregorio, B. T. Nagle, and E. K. Knaiz, *J. Dent. Res.*, **55**, 43 (1976); *b*) G. J. DiGregorio, A. J. Piraino, B. T. Nagle, and E. K. Knaiz, *ibid.*, **56**, 502 (1977); *c*) G. J. DiGregorio, A. J. Piraino, and E. Ruch, *Drug Alcohol Dependence*, **3**, 43 (1978); *d*) A. J. Piraino, G. J. DiGregorio, and E. K. Ruch, *J. Pharmacol. Method*, **3**, 1 (1980); *e*) K. R. Gans, D. J. Wood, and G. V. Rossi, *J. Pharm. Sci.*, **69**, 217 (1980);*f*) L. A. Celio, G. J. DiGregorio, E. Ruch, J. N. Pace, and A. J. Piraino, *ibid.*, **72**, 597 (1983).
6) J. Watanabe, I. Koyama, K. Iwamoto, and S. Ozeki, *J. Pharm. Pharmacol.*, **39**, 912 (1987).
7) J. Watanabe, T. Setoyama, K. Iwamoto, Y. Hayashi, and S. Ozeki, *Pharm. Res.*, **2**, 185 (1985).
8) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
9) S. B. Matin, S. H. Wan, and J. H. Karam, *Clin. Pharmacol. Ther.*, **16**, 1052 (1974).
10) A. G. Gilman, L. S. Goodman, T. W. Rall, and F. Murad (eds.), "Goodman and Gilman's The Pharmacological Basis of Therapeutics," 7th ed., Macmillan Publishing Co., New York, 1985, pp. 1267—1271.
11) G. G. Graham, *Pharmacol. Ther.*, **18**, 333 (1982).