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Dose-Dependent Salivary Excretion Following Bolus Intravenous Administration of Lithium in Dog[1)]

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Salivary excretion of lithium was investigated following bolus intravenous administration at low or high dose (0.145 or 1.45 meq/kg) to beagle dogs. Salivation was stimulated with 10% citric acid. Parotid saliva (Pr) and mandibular-sublingual saliva (MS) were collected separately by means of permanent fistulae. (1) The concentrations of lithium in both saliva and plasma declined biexponentially with time at both dose levels. (2) The lithium concentrations in each saliva were higher than and well correlated to those in plasma at both dose levels. (3) The saliva/plasma lithium concentration ratios (S/P ratios; Pr 2.19, MS 1.59) at high dose were significantly higher than those (Pr 1.64, MS 1.35) at low dose in the Pr and MS ($p<0.001$). (4) The S/P ratio and salivary clearance (CL_s) of lithium slightly increased with the plasma concentration of the drug. These results suggested that salivary excretion of lithium depended on the dose level or the plasma concentration. (5) CL_s of lithium was well correlated to that of sodium or potassium at both dose levels. The CL_s values of lithium and potassium at high dose were greater than those at low dose, but the CL_s of sodium remained unchanged at the two doses. (6) Mean total salivary clearance $[(CL_{Pr}+CL_{MS})\times 2]$ of lithium was about 70% and 60% of its total body clearance at low and high doses, respectively.

Keywords——lithium; dose-dependence; salivary drug excretion; salivary drug concentration; dog; parotid saliva; mandibular-sublingual saliva; salivary clearance; total body clearance; sodium; potassium

Monitoring of drug concentrations in blood has been widely carried out, since the importance of clinical pharmacokinetics has been increasingly recognized. However, the collection of blood samples causes pain due to the inevitable venipuncture. It is expected that the drug concentrations in saliva, which can be collected by relatively non-invasive techniques, may be used for therapeutic drug monitoring in place of the plasma concentrations. Investigations for this purpose have been carried out in humans mostly with drugs which have a rather small therapeutic index.

Plasma concentrations of lithium, an important drug for the treatment of manic depression, must be monitored because of the narrow therapeutic range (0.8—1.2 meq/l) and severe side effects. The salivary excretion of this drug in man has been reported in many papers,[4-16)] but most of them simply describe the phenomena. In these reports, from the fact that the saliva/plasma drug concentration ratios are larger than unity, it is suggested that salivary excretion of lithium may involve an active transport mechanism. However, the mechanism of its salivary excretion has not yet been clarified.

In this study, the possible factors affecting the salivary excretion of lithium were investigated in unanesthetized dogs over a wide range of plasma concentration to obtain a clue to the excretory mechanism. The blood and two different salivas from the parotid and mandibular-sublingual glands were sampled periodically following bolus intravenous administration of lithium chloride at low or high dose. The salivary flow rate, salivary pH, protein

concentration and sodium and potassium concentrations in saliva were determined to examine the effects of these factors on the salivary excretion of lithium. Furthermore, the salivary clearance (CL_s) of lithium was estimated and compared with the total body clearance.

Experimental

Materials——Lithium chloride was of analytical grade (Wako Pure Chemical Industries Ltd., Osaka, Japan). All other reagents were commercial products of analytical grade.

Animals——Three male beagle dogs weighing 8.0—12.5 kg were employed without fasting. All beagle dogs had been operated to form permanent fistulae[17] for collecting parotid saliva (Pr) and mandibular-sublingual saliva (MS) separately.

Drug Administration and Sampling of Saliva and Blood——Dogs received 0.145 meq/kg (low dose level) or 1.45 meq/kg (high dose level) of lithium chloride through the cephalic vein as an aqueous solution of 0.290 or 1.45 meq/ml, respectively.

Devices described in the previous paper[17] were used for collection of saliva. Two kinds of saliva were individually led through Tygon Tubings (i.d. 1/16 inch) and collected under a liquid paraffin layer (about 3 ml) in test tubes. Salivation was gustatorily stimulated by applying 0.4 ml of 10% citric acid onto the tongue of each dog. The protocol of saliva and blood collection is shown in Chart 1 in relation to the gustatory stimulation. The preliminary stimulations were given 2 and 5 min before the start of the saliva collection period, and saliva samples were collected for 2 min with three stimulations, every 30 s. The blood sample was taken at the midpoint of the saliva collection period. The plasma was obtained by centrifugation of the blood sample at 3000 rpm for 15 min.

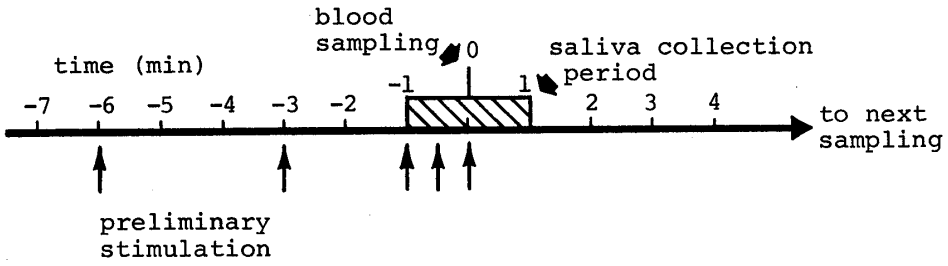

Chart 1. Protocol of Saliva and Plasma Collections

An upward arrow represents a stimulation of salivation with 10% citric acid. The blood sample was taken at the midpoint of the saliva collection period.

The experiments were carried out in duplicate at each dose level. The second administration was done in the same dog at least four weeks after the first experiment.

Measurement of Salivary Flow Rate, Salivary pH and Protein Concentration in Saliva——Salivary flow rate was determined from the weight of saliva sample assuming that the specific gravity of saliva was 1.00.[18] Salivary pH was measured with a combined electrode immersed through the liquid paraffin layer. Protein concentrations in saliva were determined by the method of Lowry *et al.*[19] using bovine plasma albumin (Fraction V, Sanko Pure Chemical Industries Co., Ltd., Tokyo, Japan) as a standard.

Determination of Lithium, Sodium and Potassium——Lithium, sodium and potassium ion concentrations in plasma and saliva were determined with a flame photometer (Shimadzu AA-630-12, Shimadzu Seisakusho Co., Ltd., Kyoto, Japan) after appropriate dilution with distilled water.

Estimation of Pharmacokinetic Parameters——The concentration of lithium in plasma declined biexponentially and the data were analyzed according to the two-compartment open model, as shown in Fig. 1. Least-squares regression analysis was used to estimate the first-order rate constants (k_{10}, k_{12}, k_{21}) and other pharmacokinetic parameters (V_1, V_2, half-life, total body clearance) from the measured plasma lithium levels.

Results and Discussion

Relationship between Lithium Concentrations in Plasma and Saliva

Lithium concentrations in plasma, Pr and MS following bolus intravenous administration at low dose (0.145 meq/kg) are shown in Fig. 1. The concentration–time curves for all three biological fluids showed biexponential declines. The concentrations in each saliva were higher than those in plasma at all sampling points. Similar results were also obtained at high

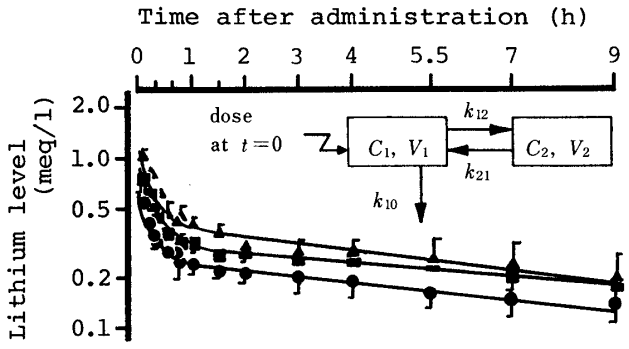

Fig. 1. Plasma and Saliva Lithium Levels Following Bolus Intravenous Administration of Lithium Chloride (Dose 0.145 meq/kg) in Three Beagle Dogs

●, plasma; ▲, Pr; ■, MS. The experiment was carried out in duplicate. Each point with a vertical bar represents the mean value ± S.D. ($n=3$—6). The solid lines for plasma, Pr and MS are the best computer-fitted curves.

TABLE I. Pharmacokinetic Parameters for Lithium Following Bolus Intravenous Administration of 0.145 meq/kg or 1.45 meq/kg of Lithium Chloride in Three Beagle Dogs

Parameters	Dose (Lithium chloride)	
	0.145 meq/kg[a] $n=65$[c]	1.45 meq/kg[b] $n=66$
A (meq/l)	0.465 ± 0.0450[d]	4.39 ± 0.665
B (meq/l)	0.256 ± 0.00936	1.89 ± 0.0749
α (min^{-1})	0.0962 ± 0.0130[e]	0.102 ± 0.178
β ($10^{-3}\,min^{-1}$)	1.40 ± 0.180[f]	1.80 ± 0.170
k_{10} ($10^{-3}\,min^{-1}$)	3.85 ± 0.495[f]	5.76 ± 0.768
k_{12} (min^{-1})	0.0587 ± 0.00941[f]	0.0664 ± 0.0143
k_{21} (min^{-1})	0.0350 ± 0.00395[f]	0.0320 ± 0.00386
V_1 (l/kg)	0.201 ± 0.0131	0.207 ± 0.0225
V_2 (l/kg)	0.337 ± 0.0696[f]	0.429 ± 0.116
$t_{(1/2)\beta}$ (h)	8.25 ± 2.91[f]	6.42 ± 0.602
CL_{tot}[g] (ml/min/kg)	0.774 ± 0.150[f]	1.192 ± 0.289

a) Weight = 1. *b*) Weight = $1/C^2$. *c*) Number of observed points. *d*) Parameter ± S.E.; W. E. Deming, "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946. *e*) Significantly different from the value at the dose of 1.45 meq/kg at $p<0.05$. *f*) Significantly different from the value at the dose of 1.45 meq/kg at $p<0.001$. *g*) Total body clearance, calculated as $k_{10} \times V_1$.

dose (1.45 meq/kg). These findings were consistent with those in man[4-16] and suggested that active transport is also involved in the salivary excretion mechanism for lithium in dogs. The estimated pharmacokinetic parameters for lithium at each dose level are shown in Table I. Almost all parameters were significantly different between these two dose levels. Although V_1 remained at almost the same level, the differences were quite large (about 50%) for k_{10} and total body clearance (CL_{tot}), which are parameters related to lithium elimination. This implies that lithium elimination from plasma is affected by the dose.

Good correlations were observed between lithium concentrations in each saliva (Pr or MS) and plasma with regard to the pooled data of the two dose levels. The correlation for Pr is shown in Fig. 2 with the correlation coefficient, r, and the regression equation, and a similar correlation was found in MS ($Y=1.78X$—0.175, $r=0.986$, $n=125$, $p<0.01$), where Y and X are the concentrations in saliva and plasma, respectively. Even in this case, however, dose-dependent salivary excretion was revealed by dividing all these data into two groups according to the dose level. The saliva/plasma drug concentration ratios (S/P ratios) at each dose level are summarized in Table II together with other saliva excretion characteristics. The S/P ratios (Pr 2.19, MS 1.59) at high dose were significantly higher than those (Pr 1.64, MS 1.35) at low dose for both Pr and MS ($p<0.001$). These results indicated that the salivary

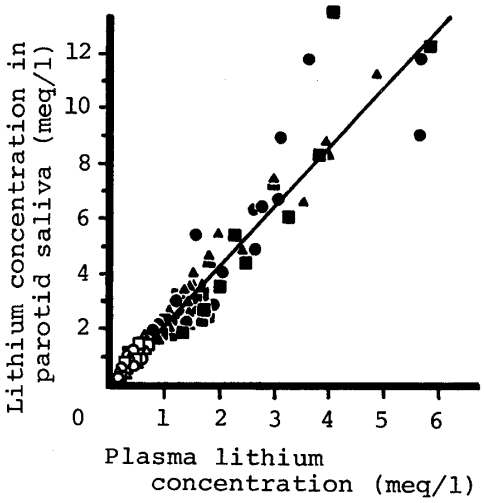

Fig. 2. Correlation between Parotid Saliva and Plasma Lithium Concentrations Following Bolus Intravenous Administration of 0.145 meq/kg (Open Symbols) or 1.45 meq/kg (Solid Symbols) of Lithium Chloride in Three Beagle Dogs

Each type of symbol represents an individual dog. The solid line shows the regression line ($Y=2.19X$, $r=0.963$, $n=129$, $p<0.01$).

TABLE II. Saliva/Plasma Concentration Ratio of Lithium (S/P Ratio), Salivary Flow Rate, Salivary pH and Protein Concentration in Saliva Following Bolus Intravenous Administration of 0.145 meq/kg or 1.45 meq/kg of Lithium Chloride in Three Beagle Dogs

		Dose (Lithium chloride)	
		0.145 meq/kg	1.45 meq/kg
S/P ratio	Pr	1.64 ± 0.24[a]	2.19 ± 0.48[b]
		$n=63$[c]	$n=66$
	MS	1.35 ± 0.14	1.59 ± 0.27[b]
		$n=60$	$n=65$
Salivary flow rate (ml/min/kg)	Pr	0.056 ± 0.026	0.063 ± 0.027
		$n=59$	$n=65$
	MS	0.130 ± 0.043	0.149 ± 0.035[d]
		$n=59$	$n=65$
Salivary pH	Pr	8.06 ± 0.06	8.06 ± 0.06
		$n=39$	$n=39$
	MS	7.84 ± 0.08	7.87 ± 0.10
		$n=60$	$n=64$
Protein conc. (mg/ml)	Pr	1.53 ± 0.86	1.15 ± 0.87
		$n=31$	$n=35$
	MS	0.90 ± 0.51	1.03 ± 0.59
		$n=34$	$n=34$

a) Mean±S.D. *b*) Significantly different from the value at the dose of 0.145 meq/kg at $p<0.001$. *c*) Number of data points. *d*) Significantly different from the value at the dose of 0.145 meq/kg at $p<0.01$.

excretion of lithium was influenced by the dose.

The relationship between S/P ratios and plasma lithium concentrations was examined to investigate the salivary excretion mechanism of lithium. A weak but statistically significant correlation was found between the S/P ratio and the plasma concentration (for Pr, $r=0.478$, $n=129$, $p<0.01$ and for MS, $r=0.550$, $n=15$, $p<0.01$). It would be difficult to explain these results only in terms of a secretory mechanism involving active transport, which has been reported in previous studies.[8,9,13,20,21] If the salivary excretion of lithium were driven only by active secretion, the S/P ratio would be almost constant or would decrease owing to saturation of the active transport with the increment of the plasma concentration.

Salivary Clearance and Plasma Concentration of Lithium

In our previous paper,[22] the concept of the salivary clearance (CL_s) was introduced to discuss the salivary excretion of a drug from a kinetic point of view. The CL_s of a drug was caluculated from the following equation,

$$CL_s = \frac{C_s \times V_s}{C_p} = [S/P \text{ ratio}] \times V_s \quad (1)$$

where C_s and C_p are the drug concentrations in saliva and plasma, respectively, and V_s is the salivary flow rate. Assuming a constant S/P ratio, CL_s is expected to be directly proportional to V_s. In fact, very high correlations were found between CL_s of lithium and salivary flow rate (at low dose: for Pr, $r=0.949$, $n=62$, $p<0.01$ and for MS, $r=0.959$, $n=59$, $p<0.01$. At high dose: for Pr, $r=0.831$, $n=66$, $p<0.01$ and for MS, $r=0.706$, $n=65$, $p<0.01$). The CL_s values obtained at each dose are summarized in Table III together with those of endogenous sodium or potassium ion. The CL_s of lithium for both Pr and MS increased significantly with the increase of dose ($p<0.001$). Furthermore, the CL_s of lithium showed a weak but statistically significant tendency to increase with the plasma concentration (for Pr, $r=0.245$, $n=128$, $p<0.01$ and for MS, $r=0.257$, $n=124$, $p<0.01$). These results also suggested that other mechanisms than diffusion may be involved in the salivary excretion of lithium. It has been reported that lithium is excreted into saliva by active secretion in the striated duct of the mandibular gland in cats.[21] In the present results, however, the salivary excretion of lithium could not be explained only by active secretion. Since higher CL_s was obtained at higher dose in this study, saturable reabsorption might be involved. Unfortunately, the rather poor relationship between CL_s and the plasma concentration of lithium because of the data fluctuation meant that we could not confirm this possibility or elucidate the characteristics of the putative saturation mechanism.

***S/P* Ratio and Salivary Flow Rate, Salivary pH or Protein Concentration in Saliva**

Burgen[20] has reported that the S/P ratio of lithium was high in the range of low salivary flow rate in parotid saliva of anesthetized dog, and that the value decreased with increase of

TABLE III. Salivary Clearance of Lithium, Sodium and Potassium Following Bolus Intravenous Administration of 0.145 meq/kg or 1.45 meq/kg of Lithium Chloride in Three Beagle Dogs

Salivary clearance (ml/min/kg)		Dose (Lithium chloride)	
		0.145 meq/kg	1.45 meq/kg
Lithium	Pr	0.090±0.039[a]	0.128±0.058[b]
		$n=62$[c]	$n=66$
	MS	0.175±0.061	0.228±0.059[b]
		$n=59$	$n=65$
Sodium	Pr	0.037±0.021	0.040±0.023
		$n=61$	$n=66$
	MS	0.087±0.033	0.094±0.028
		$n=54$	$n=65$
Potassium	Pr	0.210±0.117	0.272±0.156[d]
		$n=62$	$n=66$
	MS	0.406±0.161	0.503±0.176[e]
		$n=58$	$n=62$

a) Mean±S.D. *b*) Significantly different from the value at the dose of 0.145 meq/kg at $p<0.001$. *c*) Number of data points. *d*) Significantly different from the value at the dose of 0.145 meq/kg at $p<0.05$. *e*) Significantly different from the value at the dose of 0.145 meq/kg at $p<0.01$.

the flow rate to approach unity. On the other hand, it has been reported that the salivary flow rate had no influence on the S/P ratio of lithium in man.[10,16] In this study, negative correlations, which were similar to the results of Burgen,[20] were observed between S/P ratio and the salivary flow rate except for MS at low dose level. However, the correlation coefficients, r, were relatively low, especially at low dose level (at low dose: for Pr, $r=-0.326$, $n=62$, $p<0.05$ and for MS, $r=-0.132$, $n=59$, not significant. At high dose: for Pr, $r=-0.459$, $n=66$, $p<0.01$ and for MS, $r=-0.324$, $n=65$, $p<0.05$). The difference might be due to the fact that the present experiments were carried out at lower plasma concentration of lithium than that in Burgen's study (10.1 meq/l).[20]

As for salivary pH, no significant correlation was observed between the S/P ratio and salivary pH. Furthermore, the salivary pH remained unchanged between the two dose levels as shown in Table II. Since lithium salt is a strong electrolyte, it was not expected that the salivary pH would have any influence on the salivary excretion of lithium.

In our previous paper,[18] it was indicated that the S/P ratio of phenobarbital in dogs was influenced by salivary protein binding. However, in this study, the S/P ratio of lithium did not show any significant correlation with the protein concentration in saliva. Therefore, the S/P ratio of lithium may not be influenced by salivary protein binding. This evidence is consistent with the fact that lithium is not bound to plasma protein.[23]

Relationship between Salivary Clearance of Lithium and That of Sodium or Potassium

The salivary excretion mechanism of endogenous sodium (Na^+) or potassium (K^+) has been investigated in some detail.[24] Lithium is an alkali metal, like Na^+ or K^+. From this point of view, the salivary excretion mechanism of lithium was examined in relation to that of Na^+ or K^+ using the salivary clearance (CL_s) of these ions.

Significant correlations were observed between the CL_s values of lithium and Na^+, which is known to be reabsorbed in the striated duct,[24] for Pr and MS at both dose levels (at low dose: for Pr, $r=0.933$, $n=61$, $p<0.01$ and for MS, $r=0.900$, $n=54$, $p<0.01$. At high dose: for Pr, $r=0.701$, $n=66$, $p<0.01$ and for MS, $r=0.500$, $n=65$, $p<0.01$). The CL_s of lithium also showed quite good correlations with the CL_s of K^+ which is secreted in the striated duct[24] (at low dose: for Pr, $r=0.879$, $n=62$, $p<0.01$ and for MS, $r=0.702$, $n=58$, $p<0.01$. At high dose: for Pr, $r=0.742$, $n=66$, $p<0.01$ and for MS, $r=0.550$, $n=62$, $p<0.01$). These results suggest that the salivary excretion mechanism of lithium may have some relation to that of Na^+ and/or K^+.

The mean values of the CL_s of lithium, Na^+ and K^+ at each dose level are shown in Table III. The CL_s of lithium increased with the dose as stated before. The CL_s of K^+ showed the same tendency, but there was no difference in the CL_s of Na^+ between the two dose levels. The salivary excretion mechanism of lithium may be similar to that of K^+. Further investigation would be needed to elucidate the salivary excretion mechanism of lithium more clearly.

Total Salivary Clearance and Total Body Clearance

Total salivary clearance was calculated by doubling the sum of each salivary clearance $[(CL_{Pr}+CL_{MS})\times 2]$, since the permanent fistulae for collecting Pr and MS were made for one of each pair of these salivary glands.

The total salivary clearance of lithium was compared with the total body clearance (CL_{tot}) at both dose levels as shown in Table IV. The ratios of the total salivary clearance to CL_{tot} were high for both dose levels. Although CL_s was measured simultaneously with CL_{tot} using an identical dog in these experiments, it is considered that this CL_s would actually make little contribution to CL_{tot}. The reason is that under the conditions of these experiments, salivary excretion of lithium occurred only during very short periods of stimulated salivation and that the estimates for CL_s also corresponded only to these short periods. If salivation

TABLE IV. Total Body Clearance (CL_{tot}) and Total Salivary Clearance of Lithium Following Bolus Intravenous Administration of 0.145 meq/kg or 1.45 meq/kg of Lithium Chloride in Three Beagle Dogs

	Dose (Lithium chloride)	
	0.145 meq/kg	1.45 meq/kg
CL_{tot} (ml/min/kg)	$0.774 \pm 0.150^{a)}$ $n=65^{b)}$	$1.192 \pm 0.289^{a)}$ $n=66$
Total salivary clearance[c] (ml/min/kg)	$0.542 \pm 0.140^{d)}$ $n=56$	$0.706 \pm 0.183^{d)}$ $n=65$
Ratio of total salivary clearance to CL_{tot}	70.0%	59.2%

a) From Table I. *b*) Number of observed points. *c*) $[(CL_{Pr}+CL_{MS})\times 2]$. *d*) Mean ± S.D.

were continuously stimulated, however, the salivary excretion would contribute significantly to the elimination of lithium from the body, as expected from Eq. 1.

In this study, the following findings were obtained in relation to the salivary excretion mechanism of lithium. Salivary excretion of lithium is influenced by the plasma concentration of the drug. Salivary excretion of lithium may have some relation to that of sodium and/or potassium as regards its mechanism. However, we have not yet elucidated in detail the mechanism of salivary lithium excretion. On the other hand, from the kinetic point of view, the salivary excretion might significantly influence lithium elimination from the body. In our laboratory, further studies are in progress to examine the actual contribution of salivary clearance to total body clearance of lithium under conditions of continuous stimulation of salivation.

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