Pharmacokinetic Aspects of Therapeutic Drug Monitoring by Using Salivary Concentration: Reliability and Limitation

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Saliva sample is collected almost noninvasively and may be substituted for plasma(or serum) sample in therapeutic drug monitoring (TDM) if a consistency exists in saliva to plasma concentration ratio (S/P ratio) over a wide concentration range. Salivary therapeutic drug monitoring (STDM), therefore, offers particular advantages in geriatric and pediatric studies. However, its clinical application has been limited to several antiepileptic agents or a few other drugs having constant S/P ratio with smaller variation. This review article discusses reliability and limitation in the pharmacokinetic aspects of STDM by integrating relevant data reported previously in experimental animals and in human subjects or patients in order to focus on determining factors of S/P ratio. Salivary drug excretion across the glandular epithelial cells is essentially explained by a modified "pH-partition theory" including protein binding equilibrium. Major factors to determine S/P ratio and to affect its variation are assigned to salivary pH, plasma and saliva protein binding, lipophilicity of drug molecule and salivary flow rate. Stimulation condition influences all of these factors except lipophilicity. Kinetics of salivary drug excretion is expressed as the salivary clearance that is a product of S/P ratio and salivary flow rate. Relatively large contribution of salivary clearance to the total body clearance was estimated for phenobaribital and lithium. Pharmacokinetic and/or pharmacodynamic studies using saliva sample and clinical application have been exemplified for a number of drugs but the reliable STDM has been limited to several drugs such as phenytoin, carbamazepine, phenobarbital, primidone, digoxin or theophylline.

Keywords: salivary excretion, salivary drug concentration, therapeutic drug monitoring, saliva to plasma concentration ratio, pharmacokinetics

Introduction

Concentration of drug in the body, systemic circulation and tissue, following administration via various routes is determined by its properties in pharmacokinetic processes such as absorption, distribution, metabolism and excretion. All of these processes involve a passage of drug molecule across cell membranes. Therefore, mechanisms by which drug molecules cross membranes and the physicochemical properties of molecules and membranes that influence this transfer are critical to understanding the disposition of drug in the human body. As regards excretion of drugs the kidney is the most important organ for excreting drugs and their metabolites. Substances excreted in the feces are principally unabsorbed orally ingested drugs or drug metabolites excreted either into the bile or secreted directly into the intestinal tract and not reabsorbed. Excretion of drugs into breast milk is important not because of the amount eliminated, but because of the excreted drugs as potential sources of unwanted pharmacological effects in the nursing infant. Excretion from the lung is particularly important for the elimination of anesthetic gases. In contrast, excretion by other routes such as into sweat, saliva and tears is quantitatively unimportant. Elimination by these routes depends mainly on simple, passive diffusion of the unionized lipid-soluble form of drugs through the epithelial cells of the glands and also depends on the pH.

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Drugs excreted into the saliva enter the mouth, where they are usually swallowed down. The concentration of some drugs in saliva parallels that in plasma. In such case, saliva may be a useful biological fluid in which to determine drug concentrations when it is difficult or inconvenient to obtain blood samples.[1] Unique life science example has been first reported in "Results of The Detailed Supplementary Objectives Conducted Abroad The Space Shuttle, Apollo-Skylab 1981-1986", i.e. inflight pharmacokinetic research on acetaminophene, scopolamine and dextroamphetamine and monitoring of cortisol levels during staying in aerospace by using saliva samples.[2] Composition of whole saliva obtained under no stimulation has been conventionally utilized for monitoring physiological disorders as well as diagnosing periodontitis.[3] Furthermore, detection of xenobiotics or toxic agents in saliva specimen has been sometimes employed as a positive proof in forensic medicine or chemical jurisprudence.

Since saliva samples can be collected almost noninvasively in human studies, the therapeutic drug monitoring (TDM) by utilizing salivary data is favored in case of elderly or infant patients. The salivary excretion of drugs as xenobiotics has been the objects of numerous investigations during last three decades along with the developments in pharmacokinetics and clinical pharmacokinetics. Matin *et al*. have proposed that the saliva to plasma concentration ratio(S/P ratio) for weakly acidic or basic compounds can be predicted from a modified pH-partition hypothesis.[4] The observation that drug levels in saliva are proportional to their plasma levels has led to the suggestion that in TDM and pharmacokinetic studies saliva might be substituted for plasma. TDM by means of saliva sample, namely salivary therapeutic drug monitoring(STDM), has more significant merit that salivary drug level tends to reflect plasma unbound drug level which is in general related to the pharmacological effects than that the saliva samples are usually collected by a convenient and almost noninvasive procedure. The essential prerequisite for saliva utilization in TDM or pharmacokinetic studies is the presence of a consistent correlation between drug concentrations in saliva and plasma, namely a constant S/P ratio, over a broad concentration range.[5, 6] However, Danhof and Breimer have reported that S/P ratios for several drugs in human studies tend to fluctuate with relatively large standard deviations.[7] Furthermore, relatively large discrepancies between observed and predicted S/P ratios calculated by Matin's equation have been already reported on several drugs such as phenobarbital, 5-fluorouracil(5-FU) or mexiletine in animal experiments or clinical studies.[8-10]

In order to expand a utilization of STDM and to develop pharmacokinetic studies by using salivary drug concentrations, relevant factors to affect consistency of S/P ratio and to involve discrepancy from Matin's theory should be discussed in detail. In this paper, we explain hypothetical model of salivary drug excretion mechanism and collection methods of secreted saliva samples in the first part, and then discuss factors determining and affecting S/P ratio, kinetic aspects of salivary drug excretion, results of pharmacokinetic and pharmacodynamic studies using salivary drug concentrations and of clinical trial or application of STDM by reviewing previous reports obtained in animal experiments and human studies.

Mechanism of salivary drug excretion

The prototype model for the movement of drug molecule across the parotid glandular membrane epithelia has been first proposed by Borzelleca and Putney who employed salicylate.[11] This model is simply based on so-called pH-partition hypothesis where only unionized form of salicylate can be transferred by passive diffusion through glandular epithelial cells which behave as lipid membrane barrier to attain an equilibrium between plasma and saliva sides. Subsequently, Matin *et al*. have proposed a modified pH-partition theory by including a concept of protein binding effect in both plasma and saliva.[4] As schematically shown in Fig. 1, an equilibration is kept for unbound fraction of unionized form of drugs. They proposed Eq.1 and 2 to express the S/P ratio for weakly acidic and basic drugs, respectively.

For acidic drugs:

$$S/P\ \text{ratio} = C_s/C_p = \{(1+10^{(pHs-pKa)})/(1+10^{(pHp-pKa)})\} \times f_{up}/f_{us}$$

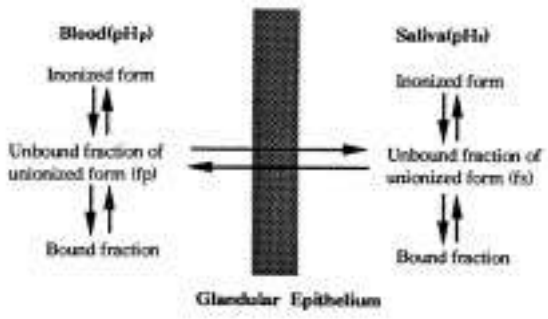


Fig.1. Schematic Representation for Transfer of Drug Molecule between Blood Plasma and Saliva Based on Modified pH-Partition Theory

(Eq. 1)

For basic drugs:

$$S/P\ ratio = C_s/C_p = \{(1+10^{(pKa-pHs)})/(1+10^{(pKa-pHp)})\} \times f_{up}/f_{us} \quad \text{(Eq. 2)}$$

where C_s and C_p are drug concentrations in saliva and plasma, respectively, pH_s and pH_p are pH values of saliva and plasma, respectively and f_{us} and f_{up} are unbound drug fractions in saliva and plasma, respectively.

These two equations are called "Matin's Equations" meaning that the S/P ratio of drugs existing as weak electrolytes in plasma and saliva is determined by p*K*a value of drug molecule, pH values of plasma and saliva, and unbound fractions in plasma and saliva. They have reported that measured value of S/P ratio for tolbutamide(p*K*a=5.4) almost coincides with calculated value according to Eq. 1.[4] Therefore, power of ionization of drug itself, salivary pH and protein binding capacity in plasma are key factors to determine the S/P ratio based on the assumption that plasma pH is maintained to be physiologically normal at pH 7.4 and protein binding of drug in saliva is almost negligible. Differences in salivary pH caused by different stimulation methods or separate collection from the individual glands are discussed below.

Collection methods of secreted saliva sample

Saliva secretion from three major glands, parotid, mandibular and sublingual glands, is known to be subject to dual controls by sympathomimetic and parasympathomimetic nervous systems, where the latter system predominates to facilitate secretion to a greater extent.[8] In general, salivary flow rate under stimulation is highest in the mandibular gland, followed by parotid gland, and lowest in sublingual gland.[8] It has been well known that salivary levels of many electrolytes including bicarbonate, a few cations and anions and of proteins as well as hydrogen ion determining pH are variable depending on stimulation conditions. In general, salivary pH rises and total protein level decreases as salivary flow increases.[10]

Stimulation is classified into chemical and physical methods. There are a few chemical, i.e. sapid, stimulation methods employed mainly in animal experiments or in a few human studies where an aliquot of acid(citric acid) or salt(sodium chloride) solution is applied on tongue right before programmed sampling timing. For STDM studies, on the other hand, collection of resting saliva by applying cotton ball as absorbent device may be most favorable because of almost noninvasive condition in clinical trials.[10] In some trials, a certain stimulation with some sapid aliquot applied on subject tongue or by constrained mumbling with or without a piece of parafilm or silicone rubber held in mouth cavity of subject may be also employed. For securing reliable data of drug concentration in saliva samples, contamination with orally ingested drug, dilution of saliva with chemical stimulant solution and adsorption of drug to physical stimulation device such as parafilm or silicone rubber should be avoided.[10] In animal experiments where saliva samples are collected separately from the individual glands, particular precaution has been taken to avoid a spontaneous elevation of collected saliva pH by alkalization as described later.

Factors determining and affecting S/P ratio

Parallel, consistent correlation between plasma and salivary drug concentrations over a broad concentration range is the essential prerequisite for utilization of saliva samples in place of plasma samples in TDM. The consistency of S/P ratio favors to encourage STDM. Danhof and Breimer have reviewed the S/P ratios for 31 drugs which were reported by different authors.[6] Coefficient of variation(C.V.) which was calculated from the data reported as the mean value and S.D. for 14 drugs is summarized in Table 1. The C.V. values from several reports were larger than 20% for digoxin, lithium, penicillin, phenobarbital, phenytoin, procainamide, quinidine, streptomycin and sulphapyridine. Some reports on penicillin and phenytoin resulted in 100% C.V. These differences in C.V. among drugs and those in each drug among reports might be both derived from that the study backgrounds and conditions including population of patients or subjects, stimulation for salivation and collection methods of saliva samples are not necessarily identical.

Matin's equations, Eq. 1 and 2, indicate that critical factors to determine the S/P ratio of the drug and to affect its fluctuation are primarily salivary pH and plasma and salivary protein binding. In addition to these factors, lipophilicity of the drug and salivary flow rate should be considered.

1) Salivary pH

Mucklow *et al*. have proposed theoretical correlation between log C_s/C_p, i.e. S/P ratio and salivary pH as shown in Fig. 2.[12] These correlations were produced from virtual salivary pH and the S/P ratio calculated by Eq. 1 and Eq. 2 by assuming plasma pH at 7.4. In

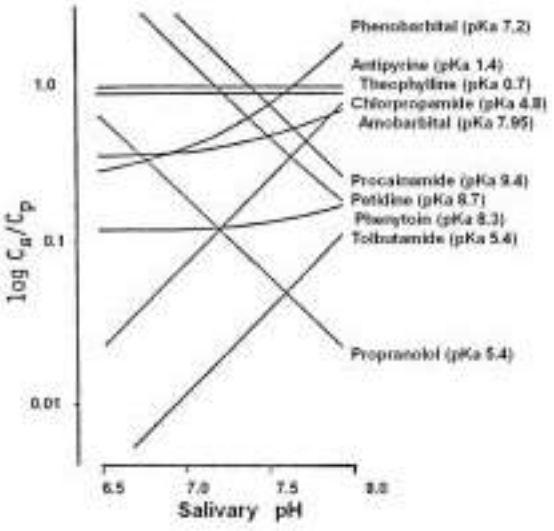


Fig.2. Theoretical Relationship between Logarithm C_s/C_p and Virtual Salivary pH for 10 Model Drugs

The C_s/C_p ratio for each acidic or basic drug was calculated according to relevant Matin's equation where pH 7.4 for pH_p, each p*K*a value, reported plasma unbound fraction for f_p and assumption of 1.0 for f_s were employed. (Ref. 12)

theory, positive correlation of log(Cs/Cp) against virtual salivary pH is obtained for acidic drugs, while negative correlation for basic drugs. They have further proposed that reliable prediction of S/P ratio may be obtained especially for weakly acidic and basic drugs which are largely nonionized at normal plasma pH, such as phenytoin, phenobarbital and antipyrine, but unreliable for ionized drugs at normal plasma pH, such as chlorpropamide,tolbutamide,propranorol and meperidine.[12] Mixed whole saliva is usually collected in response to light mechanical mouth movement or to sapid stimuli in human studies where measured salivary pH value itself is easily subject to spontaneous change by alkalization.

Modulation of salivary pH by changing stimulation condition and determination of glandular difference in salivary pH and S/P ratio have been systematically investigated by Watanabe *et al.* in rats, rabbits and dogs. In these animal experiments, each saliva sample from major salivary glands, Pr and M(or MS in case of beagle dogs) gland, was separately collected through polyethylene (PE-10 for rats, PE-50 for rabbits) or Tygon(for beagle dogs) tubing under liquid paraffin

Table 1. Reported Mean Value with Standard Deviation(S.D.) and Calculated Coefficient of Variation(C.V.) of Saliva/Plasma Concentration(S/P) Ratio for 14 Drugs

Drug	Mean S/P Ratio ± S.D.	C.V. (%)	Originally Reported by
Acetazolamide	0.009 ± 0.001	11.1	Wallace *et al.*(1977)
Aminopyrine	0.79 ± 0.04	5.1	Vesell *et al.*(1975)
Carbamazepine	0.26 ± 0.01	3.8	Westenberg *et al.*(1977)
	0.42 ± 0.05	11.9	Bartels *et al.*(1977)
Digoxin	1.14 ± 0.48	42.1	Jusko *et al.*(1975)
	0.78 ± 0.07	9.0	Huffman(1975)
	1.34 ± 0.44	32.8	Joubert *et al.*(1976)
Lithium	2.85 ± .59	20.7	Groth *et al.*(1974)
Penicillin	0.015 ± 0.015	100.0	Bender *et al.*(1953)
Pentobaribital	0.42 ± 0.18	42.9	Breimer *et al.*(1976)
Phenytoin	0.103 ± 0.015	14.6	Paxtone *et al.*(1977)
	0.11 ± 0.02	18.2	Horning *et al.*(1977)
	0.24 ± 0.24	100.0	Bochner *et al.*(1974)
	0.108 ± 0.051	47.2	Barth *et al.*(1976)
Procainamide	3.50 ± 2.34	66.9	Koup *et al.*(1975)
	1.62 ± 0.61	37.7	Galezzi *et al.*(1976)
Qunidine	0.51 ± 0.12	23.5	Jaffe *et al.*(1975)
Streptomycin	0.15 ± 0.08	53.3	Bender *et al.*(1953)
Sulphanilamide	0.87 ± 0.10	11.5	Killmann *et al.*(1955)
Sulphapyridine	0.81 ± 0.10	21.0	Killmann *et al.*(1955)
Theophylline	0.52 ± 0.03	5.8	Koysooko *et al.*(1974)
	0.77 ± 0.07	9.1	Koup *et al.*(1975)
	0.48 ± 0.04	8.2	Shah and Riegelman(1974)

The C.V. value was calculated from the mean and S.D. reported by Danhof and Breimer(Ref. 7).

Table 2. S/P Ratio and Its Glandular Difference for Several Drugs in Experimental Animals

Drug (Dose)	Animal	Stimulant	S/P Ratio, Mean ± S.D.	
			Pr	M or MS
Phenobabital (10 mg/kg)	Dogs	Acid	0.923 ± 0.175	0.633 ± 0.073
		Salt	0.597 ± 0.071	0.509 ± 0.067
Phenytoin (10 mg/kg)	Dogs	Acid	0.271 ± 0.070	0.220 ± 0.062
		Salt	0.260 ± 0.076	0.232 ± 0.060
5-Fluorouracil (20 mg/kg)	Dogs	Acid	0.472 ± 0.303	0.200 ± 0.196
Lithium				
(0.145 mEq/kg)	Dogs	Acid	1.64 ± 0.24	1.35 ± 0.14
(1.45 mEq/kg)		Acid	2.19 ± 0.48	1.59 ± 0.27
Indomethacin				
(20 mg/kg)	Dogs	Acid	0.074 ± 0.034	0.044 ± 0.024
(15 mg/kg)	Rabbits	Pilocarpine	0.0151 ± 0.0119	0.0608 ± 0.0102
Procainamide (50 mg/kg)	Rats	Pilocarpine	0.974 ± 0.243	0.284 ± 0.119

Acid: 10% citric acid solution, Salt: 15% NaCl solution. (Ref. 10).

Table 3. Comparison of Measured S/P Ratio with the Theoretical Ratio in Mandibular Saliva for Several Drugs or Chemical Compounds Administered to Dogs under Stimulation with 10% Citric Acid

Drugs or Chemicals	Measured S/P Ratio		Theoretical S/P Ratio[a)]	
	Mean ± S.D.	(n)[b)]	Mean ± S.D.	(n)
Indomethacin	0.044 ± 0.024	(55)	0.053	(1)
Phenobarbital	0.648 ± 0.088	(41)	1.09 ± 0.132	(33)
5-Fluorouracil	0.200 ± 0.196	(31)	1.59 ± 0.130	(31)
Phenytoin	0.220 ± 0.062	(35)	0.235 ± 0.024	(35)
Verapamil	0.0870 ± 0.0435	(29)	0.0516 ± 0.0159	(29)
Lithium	1.35 ± 0.14	(60)	NC[c)]	
Urea	0.454 ± 0.117	(40)	1.03 ± 0.000	(40)
Creatinine	0.0398 ± 0.0099	(27)	1.00 ± 0.000	(31)

a)Calculated by Matin's equations(Eq. 1 and Eq. 2). b) Number of available data points. c)Not able to be calculated. (Ref. 10).

layer to avoid spontaneous alkalization before subsequent pH measurement by an electrode. Table 2 summarizes the S/P ratios for phenobarbital, phenytoin, 5-FU, lithium, indomethacin and procainamide in different animal species in which each saliva was separately collected under different stimulation conditions.[13)-18)] In beagle dogs under the stimulation with acid or salt, MS salivary pH is or tends to be lower than Pr salivary pH. Contrariwise in rats and rabbits under the stimulation with pilocarpine given via intravenous(i.v.) infusion and subcutaneous injection, respectively, the salivary pH values in M saliva are or tend to be higher than those in Pr saliva. Based on pH-partition theory, weakly acidic drugs such as 5-FU are hypothesized to show lower salivary drug concentration and S/P ratio at lower salivary pH, while weakly basic drugs such as procainamide are hypothesized to show lower salivary drug concentration and S/P ratio at higher salivary pH. These hypotheses were systematically certified in different animal species under different salivation conditions with different stimuli by Watanabe *et al.* as shown in Table 2 where glandular difference in S/P ratio is also relevant to the difference in salivary pH for several drugs[13)-18)]. The glandular difference in S/P ratio for all of these drugs except lithium which is neither acidic nor basic is totally explained by pH-partition hypothesis. However, the measured, i.e. observed S/P ratio particularly for phenobarbital and 5-FU in beagle dogs was substantially smaller than the theoretical ratio calculated from Matin's equation as shown in Table 3. There may

be two possibilities for these discrepancies between measured and theoretical S/P ratios. One is a critical difference of measured salivary pH from the virtual pH, i.e. the pH of primary saliva in the acinus. For procainamide though the discrepancy of which was rather small in rats,[18] intracellular pH of the rat mandibullar gland cells, which was estimated according to Borzelleca's model,[11] predicted saliva to venous effluent concentration ratio closer to the observed ratio than salivary pH did when the gland was *in situ* perfused with Krebs-Ringer bicarbonate buffer solution adjusted at pH 7.4 and 8.0.[19] The other possibility may be an involvement of factors other than salivary pH to modulate the S/P ratio.

Deliberate alteration of saliva flow rate and pH using different stimuli on different glands may produce substantial changes in salivary drug concentration, i.e. S/P ratio.[12] Wide inter-individual variability of salivary pH induced by altered flow rate may be the likely explanation for the inconstancy of S/P ratio for ionized drugs. Increase in salivary pH has been reported to be associated with increased saliva flow rate under stimulation.[20] Flow rate dependent excretion of physiological electrolytes such as sodium, potassium, calcium and inorganic phosphate has been reported in both parotid and mandibular saliva after stimulation with pilocarpine and acidic beverage to human volunteers[21, 22] and pH and bicarbonate excretion in the rat parotid gland as a function of salivary flow rate were also reporetd.[23] Therefore, it is considered that under a certain stimulation condition for salivation, salivary flow rate and salivary pH are mutually and complicatedly associated to modulate or change the salivary excretion of drug, involving large fluctuation of the S/P ratio or its discrepancy from the theoretical value calculated by Matin's equation.

2) Plasma and salivary protein binding

Based on Matin's equation, unbound fraction of drug in plasma, f_{up}, is another important factor to determine the salivary drug concentration, C_s, and S/P ratio. Saliva drug level has been reported to precisely or approximately reflect the plasma unbound fraction of drug in human subjects for dapson,[24] fleroxacin,[25] gatifloxacin[26] or moxifloxacin[27] and in animal model such as rats or rabbits for warfarin,[28] mexiletine[29] or diazinon.[30] Drug interaction on plasma protein binding between coadiministered drugs and substantial change in plasma protein level induced by some disease states such as hepato-renal failure may directly affect unbound fraction of drug in plasma involving distinct change in the S/P ratio.

Since total protein content in saliva is essentially so low as almost negligible compared to the plasma protein concentration, i.e. approximately 2.5 to 5% of that in plasma, unbound fraction of drug in saliva, f_{us}, in Eq. 1 to 2 has been usually assumed to be 1.0.[31] Salivary protein level is considered to be modulated by stimulation condition. There are a few investigations on salivary protein level and the S/P ratio of drug associated with stimuli.[13, 14, 16] Phenobarbital is one of the drugs relatively highly bound to plasma and saliva protein. Watanabe *et al*. have reported that S/P ratio of phenobarbital in Pr saliva was larger under citric acid stimulation than under salt stimulation applied to beagle dogs, being associated with higher protein level in the saliva as shown in Fig. 3 where similar but less pronounced trend was found in MS saliva.[13] For both Pr and MS, S/P ratio was found to be correlated with salivary protein level. Similar result is shown in Fig. 4 where, in two healthy volunteers, the S/P ratio and protein level of whole mixed saliva were compared between two different stimulations with citric acid and

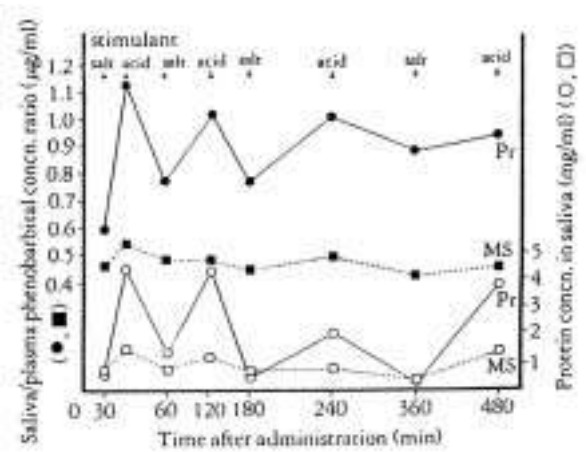


Fig.3. S/P Ratio of Phenobarbital and Salivary Protein Level in Beagle Dogs

Phenobaribital was administered intravenously with a bolus dose of 5 mg/kg. Periodical parotid(Pr: closed or open circle) and mandibular-sublingual(MS: closed or open square) saliva samples were collected separately via the permanent fistula from each gland. Salivation was stimulated alternately with 10% citric acid(acid) and 15% NaCl(salt) solution an aliquot of which was applied onto the tongue. Salivary protein level is expressed as open symbol. (Ref. 13).

constrained mumbling with silicone rubber piece in place of with salt.[10] Similar cases may be supposed to happen for other drugs which are *a priori* bound to plasma protein to higher extent. From *in vitro* study, Pohto reported that about 50% of salicylic acid at low concentration was bound to human whole-mouth saliva and the degree of binding was dependent on the drug concentration.[32]

3) Lipophilicity of drugs

Matin's equations, Eq. 1 and 2, based on pH-partition theory hypothesize that only unionized, unbound fraction of drugs can be excreted into saliva on keeping equilibrium attained between plasma and saliva sides(Fig.1). This hypothesis is considered to be applicable to drugs with moderate lipophilicity, but not to those with extremely low or high lipophilicity which are difficult to be steadily equilibrated between both sides. In case of those drugs, there may be large discrepancy of the measured S/P ratio from the theoretical ratio calculated by Matin's equation. For several drugs, Hayashi and Watanabe have estimated excessive fraction(EF) value that is percentage of fraction of difference in measured from calculated S/P ratio to measured ratio and found that EF values are concavely related with apparent lipid partition coefficients as shown in Fig. 5.[33]

On the other hand, there is a case necessary to modify Matin's equation. Fluoroquinolones which are known to exist as zwitterionic forms at physiological pH(Fig. 6)[34] behave electrically neutral there and can primarily diffuse across the plasma to salivary gland cell membrane.[35), 36] Li *et al*. have compared the measured saliva to plasma unbound concentration(P_u) ratios(S/P_u ratios)for five fluoroquinolones, ciprofloxacin(CPFX), norfloxacin (NFLX), lomefloxacin(LFLX), ofloxacin(OFLX) and sparfloxacin(SPFX), with the theoretical ratios for these fluoroquinolones in rats and then investigated a dependency of these ratios on their lipophilicities.[36] They have proposed that the theoretical S/P and S/P_u ratios can be expressed as the following equation by modifying the Matin's equation.

$$S/P \text{ ratio} = \{(1+10^{(pK_1-pHs)}+10^{(pHs-pK_2)})/(1+10^{(pK_1-pHp)}+10^{(pHp-pK_2)})\} \times f_{up}/f_{us} \quad \text{(Eq. 3)}$$

$$S/P_u \text{ ratio} = S/P \times 1/f_{up} \quad \text{(Eq. 4)}$$

where f_{us} for fluoroquinolones was assumed to be 1.0.[31]

Measured S/P_u ratios were compared with the theoretical ratios for five fluoroquinolones as shown in Table 4. In contrast to the measured ratios possessing some significant difference or its tendency between Pr and M saliva according to their pH differences, the theoretical ratios of the five fluoroquinolones were among almost 1.0 to 1.3 without any obvious difference in the ratios between both salivary glands. This means that salivary distribution of these agents cannot be explained solely by the pH-partition theory. On the other hand, the

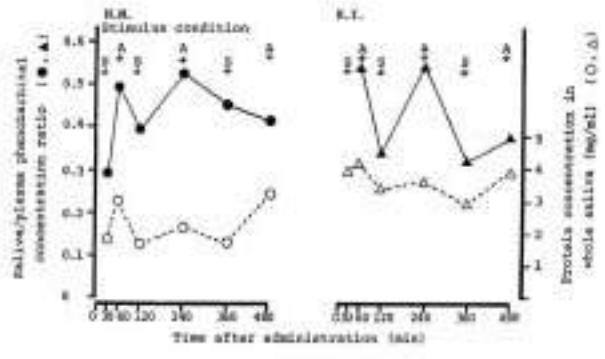


Fig.4. S/P ratio of Phenobarbital and Salivary Protein Level in Human Subjects

Phenobarbital was administered orally to two healthy male volunteers(N.M.and K.I.) at a dose of 0.5 mg/kg. Whole mixed saliva(closed symbol) sample was collected periodically from the mouth cavity. Salivation was stimulated alternately with 10% citric acid(A) applied onto the tongue and by constrained mumbling with a piece of silicone rubber(S). Salivary protein level is expressed as open symbol. (Ref. 10)

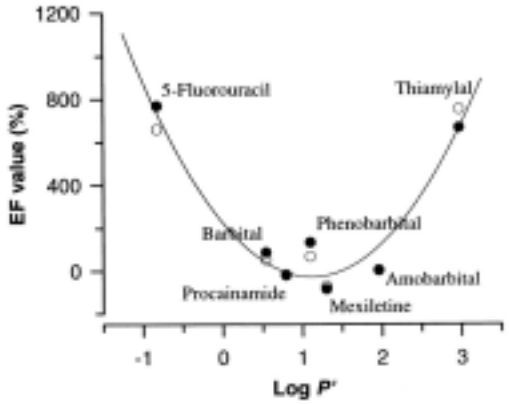
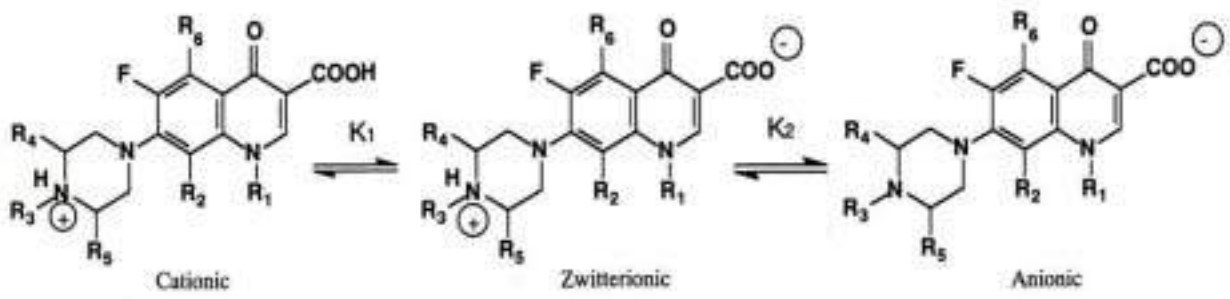


Fig.5. Relationship between Apparent Partition Coefficient(P') and Excessive Fraction(EF) Value of S/P Ratio for Several Drugs in Rats

Pr saliva: closed circle. M saliva: open circle. P' was determined by *n*-octanol and water partition system. EF value(%) was estimated as (calculated S/P − measured S/P)／measured S/P. Regression curve is expressed as $Y=225X^2-452X+202$, n=14, r=0.970, $p<0.01$. (Ref. 33).



Compound	R1	R2	R3	R4	R5	R6	pK1	pK2
NFLX	C_2H_5	H	H	H	H	H	6.30	8.38
CPFX		H	H	H	H	H	6.09	8.74
LFLX	C_2H_5	F	H	H	CH_3	H	5.82	9.30
OFLX	-(CH_3)CH-CH_2-O-	CH_3	H	H	H	H	6.05	8.22
SPFX		F	H	CH_3	CH_3	NH_2	6.23	8.57

Fig.6. Chemical Structure and p*K* Values of Fluoroquinolones
The pK_1 and pK_2 values were cited from the paper of Furet *et al*. (Ref. 34).

measured S/P_u ratios showed a positive linear relationship against the apparent partition coefficient(P') of these fluoroquinolones determined by *n*-octanol-water(pH 7.4 phosphate buffer solution) partition system as indicated in Fig. 7, suggesting that the lipid solubility may be one of the other determinants for penetrability of quinolones into saliva than those defined in modified Matin's equation.

For barbiturates, it has been also shown that the salivary excretion of five derivatives, as indicated by their S/P ratios, can be correlated with the lipid solubility as measured by the lipid/water partition coefficient, whereas it can be correlated to a lesser extent with the degree of drug ionization and plasma protein binding.[37] Therefore, it may be suggested that there is a certain dependency of S/P ratio on the lipophilicity among drugs at least belonging to the homologous derivatives.

4) Salivary flow rate

The fourth factor which may influence the S/P ratio of drugs or chemical compounds is salivary flow rate. For example, urea is one of the unionized, neutral compounds and therefore is considered to give strictly the theoretical value of 1.0 for its S/P ratio. It is true that the S/P ratio of exogenous urea shows almost 1.0 where the salivary flow rate is extremely low or extrapolated to approximately zero, but it decreases in an inversely proportional fashion as an increase of salivary flow rate.[38] This tendency has been involved in the similar disparity resulted between measured and theoretical ratios for other drugs or compounds in human subjects and other animal species. This suggests that concentration equilibrium for unionized, unbound fraction between plasma and saliva sides may be always modulated by salivary flow rate. Some phase may be involved for equilibrium to be neither steadily nor necessarily attained depending on the flow rate. Kamali and Thomas have demonstrated that salivary phenytoin concentration is increased by reductions in saliva flow rate induced by atropine in randomized placebo-controlled crossover study among epileptic patients.[39]

5) Other factors

Glandular difference and stereoselectivity are other possible factors and the former has been described above in the parts of salivary pH and salivary protein binding. There are a few interesting reports on stereoselectivity in salivary excretion. For amphetamine

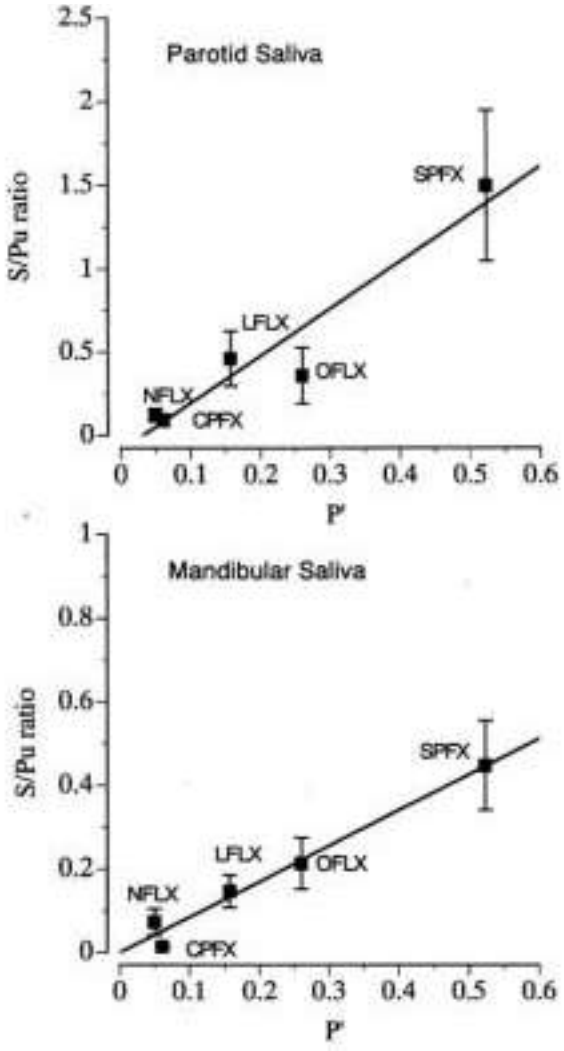


Fig.7. Relationship between Apparent Partition Coefficient(P') and Measured Saliva/Plasma Unbound(S/P_u) Ratio of Fluoroquinolones in Rats

P' was determined by *n*-octanol and water(pH 7.4 phosphate buffer solution) partition system. Each point and vertical bar represents the mean and S.D. of 3 to 4 rats. The straight lines were obtained by linear regression analysis. (Ref. 36)

administered in healthy subjects, salivary level was higher than the plasma level and S(+)-isomer was excreted into saliva more extensively and rapidly than R(-)-isomer.[40] Similar tendency has been reported for mexiletine isomers in healthy subjects, indicating that the overall mean saliva to serum free mexiletine enantiomer area under the concentration-time curve ratio is higher for S(+)-isomer than R(-)-isomer and the overall mean saliva S(+)-isomer concentration is also higher than the R(-)-isomer.[41] These stereospecific differences may be explained by stereoselective difference in the binding to plasma or serum proteins.[41]

Kinetics of salivary drug excretion

Kinetic assessment of salivary drug excretion has been first proposed as a concept based on the same consideration as tissue clearance or total body clearance by Graham.[42] Proposed concept for salivary clearance, Cl_s, is namely an integration of S/P ratio and salivary flow rate(V_s) as simply described as follows:

CL_s=Salivary drug excretion rate/C_p=($C_s \times V_s$)/C_p=(C_s/C_p) $\times V_s$=(S/P)$\times V_s$ (Eq. 5)

Salivary clearance is, therefore, expressed as a product of S/P ratio and V_s, where if the S/P ratio is kept constant, salivary drug clearance is directly proportional to the V_s. This kinetic assessment has been performed in order to know the contribution of CL_s by whole salivary glands, i.e. Pr and MS in dogs or Pr and M in rats, to total body clearance(CL_{tot}) for several drugs and a few compounds, the salivary excretion data of which have been previously reported. Table 5 summarizes whole salivary CL_s and its percentile in CL_{tot} for several drugs and a few compounds in beagle dogs under acid stimulation for salivation. Contribution of CL_s to CL_{tot} was found to be almost negligible for 5-FU, creatinine, verapamil, ibuprofen and ketoprofen, while rather large to significant contribution was estimated for exogenous urea(17.8%), lithium (70%) and phenobarbital (247%).[8)-10)] Drugs or compounds with large to significant contribution of CL_s to CL_{tot} may be disposed for detoxication by means of facilitated salivation under intercepted entero-salivary circulation. Lithium is known to be readily distributed to organ tissues to higher extent and hardly detoxicated by disposition from the body, showing relatively long half-life($t_{1/2}$). As indicated in Table 2, lithium has the S/P ratio significantly larger than 1.0 and therefore is proposed to be excreted into saliva via an active transport process rather than passive diffusion based on pH-partition theory which has been applied to a number of other drugs. Watanabe *et al*. have succeeded to increase CL_{tot} of lithium to about 140% as well as the five-fold increase in CL_s by enhancing the salivation in beagle dogs co-administered with NaCl.[43]

Salivary clearance may compensate some part of reduced major systemic clearance, i.e. hepatic or renal clearance, for drugs which are eliminated exclusively via hepatic metabolism or renal excretion under the diseased states in these organs. One of the typical examples in this aspect is reported in rats with renal failure where CL_s of OFLX increased in spite of the decrease in CL_{tot}.[35), 44)]

Table 4. Measured and Theoretical S/P_u Ratio of Fluoroquinolones after Bolus i.v. Administration (10 mg/kg) in Rats (n=4)

Fluoroquinolone	Measured S/P_u Ratio		Theoretical S/P_u Ratio[a)]	
	Mean± S.D.		Mean± S.D.	
	Pr	M	Pr	M
CPFX	0.091± 0.038	0.014± 0.003*	1.003± 0.028	1.027± 0.015
NFLX	0.121± 0.038	0.073± 0.032	1.281± 0.134	1.146± 0.055
LFLX	0.461± 0.166	0.143± 0.038*	1.001± 0.002	1.018± 0.008
OFLX	0.358± 0.169	0.213± 0.061	1.142± 0.054	1.253± 0.026
SPFX	1.497± 0.450	0.447± 0.107**	1.095± 0.167	1.145± 0.053

a)Calculated by Eq. 3 and Eq. 4. Siginificant difference detected between Pr and M saliva(*P<0.05 and **P<0.01). (Ref. 36)

Table 5. Comparison of Whole Salivary Clearance and Total Body Clearance(CL_{tot}) for Several Drugs and Chemicals Administered in Dogs under Stimulation with 10% Citric Acid

Drugs or Chemicals	A:Whole Salivary Clearance[a)] mL/min/kg	B: CL_{tot} mL/min/kg	C: (A/B)× 100 % [b)]
Phenobarbital	0.442	0.179	247
5-Fluorouracil	0.129	30.6	0.423
Phenytoin	0.152	3.47	4.38
Verapamil	0.050	28.4	0.176
Lithium	0.524	0.774	70.0
(R)-Ibuprofen	0.0056	6.47	0.085
(S)-Ibuprofen	0.0061	1.43	0.505
(R)-Ketoprofen	0.0307	21.6	0.142
(S)-Ketoprofen	0.0278	1.97	1.41
Urea	0.300	1.69	17.8
Creatinine	0.030	5.03	0.596

a) Calculated by means of $(CL_{Pr}+CL_{MS})\times 2$. b) Percent for whole salivary clearance to contribute to CL_{tot}.(Ref.10).

Pharmacokinetic and pharmacodynamic studies using salivary drug concentration

Saliva sample has been tried to use in place of blood(plasma or serum) sample in pharmacokinetic and pharmacodynamic studies on various drugs frequently in human subjects or rarely in experimental animals. Estimation of pharmacokinetic parameters and assessment of pharamacodynamic profiles by using salivary drug concentration may facilitate many areas of relevant research which has been limited by the difficulty of obtaining serial blood sampling. The $t_{1/2}$, apparent volume of distribution (V_d) and CL_{tot} of antipyrine estimated from saliva were not significantly different from those estimated using plasma in healthy subjects.[45] From the analysis on procainamide in human subjects, the kinetics of drug concentration in saliva as a different compartment from plasma and of the pharmacologic effect, prolongation of the QT interval, revealed to be indistinguishable, leading to a consideration that both kinetic measurements are different from those of concentrations in plasma.[46] Thus, in normal subjects under the study condition, saliva concentrations more precisely indicated the time-course of drug at a cardiac site of action, although they did not parallel plasma drug concentrations until 6 hr or more after a rapid i.v. injection.[46] Pemoline given orally as a single dose to healthy volunteers showed the salivary elimination $t_{1/2}$ comparable with that estimated from plasma elimination curves, although the saliva drug concentrations were about 50% lower than the corresponding plasma concentrations during the elimination phase.[47]

Kinetics of drug interaction in plasma protein binding for dapsone with pyrimethamine[24] and for phenytoin with

valproic acid[48] in normal subjects were discussed for salivary drug levels to be favored in precise prediction of pharmacokinetic parameters estimated from plasma unbound concentrations. The influence of the genetically controlled deficiency in debrisoquine hydroxylation on antipyrine metabolite formation was studied in both extensive and poor metabolizers of debrisoquine by using saliva concentration time data for pharmacokinetic analysis, suggesting that a different species of the drug-oxidizing enzymes may be involved in the metabolism of both drugs.[49] Graham has suggested that the measurement of salivary concentrations is of most value particularly in introductory pharmacokinetic and bioavailability studies on compounds which is little ionized at physiological pH values including the weakly acidic drugs such as phenytoin and sulphapyridine, and the weakly basic drugs such as carbamazepine and antipyrine.[50] This suggestion was supported by Danhof *et al*. for the kinetics of antipyrine metabolism based on the salivary data.[51]

Relationship between pharmacological effect and salivary pharmacokinetics has been also reported for warfarin in rabbits, suggesting that salivary warfarin concentration which is correlated with pharmacological effect has a possibility of utilization in pharmacokinetic studies and TDM.[28] For oral anti-malarial agent, proguanil, time to peak plasma concentration and elimination $t_{1/2}$ derived from salivary levels were reported to be in agreement with values previously reported for this drug using plasma level data.[52] Griener *et al*. have demonstrated that simple noninvasive methodologies using saliva samples appear to be well suited for studying acetaminophene disposition kinetics, i.e. apparent $t_{1/2}$, V_d and CL_{tot}, in population of developmentally disabled individuals with Down's syndrome.[53] Metabolite kinetics for zwitterionic fluoroquinolone, ciprofloxacin, and distribution into saliva were reported to be affected in accordance to the kinetics of those found in plasma of healthy volunteers under the coadministration with probenecid in multiple dose which prolonged the elimination $t_{1/2}$, decreased urinary recovery, and CL_{tot} as well as renal clearance, but there was no direct effect of probenecid on salivary excretion.[54] Benetello *et al*. have reported that any significant differences are not seen in gabapentin disposition kinetic parameters in serum or saliva of epileptic patients between dosing at fasting condition and after the high-protein meal.[55] Higher concentration of spiramycin achieved in respiratory tract and saliva than in serum of pediatric patients was suggested to be related to more prolonged post-antibiotic effect against Gram-positive *cocci* of this antibiotics than erythromycin which is less penetrated and distributed into alveolar and macrophages.[56] Gastrointestinal transit characteristics of oral patch preparation was evaluated by using caffeine as a model drug in human volunteers where salivary caffeine excretion rate, rather than the mean residence time(MRT) itself or MRT minus first-appearance time into the saliva, was better utilized for pharmacokinetic analysis in the early stages of formulation development.[57]

Clinical trial or application of STDM

Antiepileptic drugs such as phenytoin and phenobarbital have been particularly favored to use as model drugs in clinical trial and application of STDM for long time. Thus there are a number of reports on their salivary concentrations aimed to utilize in TDM. Reynold *et al*. have demonstrated that salivary phenytoin concentration correlates closely with the plasma free concentrations in both patients with epilepsy and chronic renal failure on long-term therapy(Fig. 8).[58] Using a selection of weakly acidic and basic drugs, Mucklow *et al*. have suggested that the reliable prediction of plasma drug concentrations is made from saliva levels for phenytoin, phenobarbital or antipyrine, but is not made for ionized drugs such as chlorpropamide, tolbutamide, propranolol or meperidine.[12] They have further shown that a good management to monitor the salivary levels for phenobarbital and phenytoin in place of plasma concentration in pediatric patients treated against recurrent febrile convulsion.[59] Miles *et al*. have concluded from their study on three antiepileptic agents in healthy volunteers that intra-individual variability of S/P or S/P_u ratio for carbamazepine, phenobarbital and phenytoin is well acceptable for clinical monitoring and this is not a factor that should dissuade clinicians from saliva for the therapeutic monitoring of these agents.[60] Similarly, studies in children uniformly recommended saliva sampling for therapeutic monitoring of carbamazepin, phenobarbital and phenytoin, and studies in infants to children have also reported that the STDM of ethosuximide, primidone, digoxin, theophylline and caffeine is promising.[61] Recently one unique and interesting report has been found in the title "Feasibility and acceptance of salivary monitoring of antiepileptic drugs via the US Postal Service" where transit in the mail of frozen saliva samples after home collection from pediatric and adult patients over one hundred in total number for their TDM does not adversely

affect accuracy of antiepileptic drug measurement.[62] This kind of home monitoring system has been expected to expand wider in the USA because of cost-effectiveness as well as feasible acceptance of STDM.

Juntunen-Backman *et al*. have demonstrated that the measurement of salivary theophylline concentration, when the salivation is not stimulated, is reliable to predict the serum drug level after the use of slow-release preparation, "Theo-dur", in asthmatic children.[63] Onyeji *et al*. have suggested the saliva levels of anti-malarial drug, proguanil, may be useful in the therapeutic monitoring of this drug in addition to the determination of pharmacokinetic parameters.[52] Katagiri *et al*. have reported higher concentrations of mexiletine in saliva than those in serum as well as good correlation between the saliva and serum concentrations during the post-absorption phase in normal healthy volunteers.[64] They have also suggested that it may be promising to estimate the steady-state trough serum levels of mexiletine and its elimination kinetic parameters in arrhythmic patients treated longer than 2 weeks from the salivary drug levels by using the initial saliva to serum ratio on day 1. In addition, they succeeded to design individualized dosage regimen for maintaining enough trough levels of this antiarrhythmic agent in order to reduce the frequency of ventricular premature contraction and/or ventricular tachycardia to a controlled value.[65]

Bressole *et al*. have reported that a detectable amount of doxorubicin (DOX) and its metabolite, doxorubicinol (DOXol), excreted into parotid saliva after the administration to patients with various advanced neoplastic diseases may play a role in causing stomatitis as an adverse event

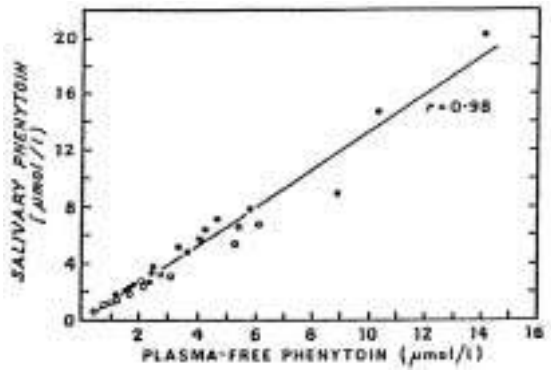


Fig.8. Correlation between Plasma Free Level and Salivary Level of Phenytoin in Epileptic Patients and Patients with Chronic Renal Failure under Long Term Therapy
Data in epileptic patients and in patients with chronic renal failure are expressed as closed and open circle, respectively. (Ref.58).

involved at relatively early stage following the chemotherapy with this agent, but failed to predict the levels of free DOX and DOXol in plasma from the S/P ratio due to huge inter-individual and pronounced intra-individual differences.[66] Recent paper on cytosine arabinoside given by infusion based on high dose regimen to the patients with refractory hematological malignancies has also shown a detectable level of the drug in saliva within 15 min after the completion of infusion, which is equivalent to 5% of its plasma concentration.[67] Salivary excretion of these anticancer agents which may cause an exposure of buccal mucosal tissue to the agents is considered to be a direct, major cause of stomatitis as one of the typical adverse events involved in the early stage during chemotherapy. Therefore, a detection of salivary levels of anti-cancer agents may be utilized for monitoring of adverse events related to stomatitides.

Greenberg *et al*. have compared the concentration of nicotine and its major metabolite, cotinine, in the saliva and the urine between infants with household exposure to tobacco smoke and the unexposed infants, showing that the concentrations in both saliva and urine are significantly higher in those from exposed group than from the unexposed group but urinary cotinine/creatinine ratio is more reliable measure of such exposure in infants.[68] On the other hand, saliva is an alternative biological matrix for drugs-of-abuse testing that offers the advantages of noninvasive, rapid and easy sampling. Measurement of 3,4-methylenedioxymethamphetamine(MDMA) in saliva has been shown to be a valuable alternative to determination of plasma drug concentration in both clinical and toxicologic studies, facilitating on-site testing by noninvasive and rapid collection of salivary specimens.[69]

Conclusion

Reviewing a number of reports from systematic studies on salivary excretion of drugs performed in both human and experimental animals has revealed hypothetical mechanism of transfer of drug molecule from blood plasma to saliva, factors affecting the S/P ratio, pharmacokinetic and pharmacodynamic data obtained by using saliva samples, and several typical cases as clinical trial and application of STDM. The essential prerequisite for saliva utilization in TDM is the presence of a consistent correlation between drug concentrations in saliva and plasma, namely a consistency in the S/P ratio, over a broad range of the concentration. However, it was concluded that reliable utilization of saliva drug concentrations for TDM has been still

relatively limited to some particular drugs including several anti-epileptic agents such as phenytroin, phenobarbital, carbamazepine, ethosuximide, primidone or valproic acid and a few other drugs such as digoxin, theophylline or caffeine. In general, salivary excretion of drugs is explained by pH-partition theory applied to unbound fraction of drug in plasma, so the primary determinants governing the potential utility of STDM are the difference between pKa value of drug molecule and salivary pH and the extent of plasma protein binding. Other possible determinants are found to be saliva protein binding, lipophilicity and salivary flow rate. In order to establish a reliable STDM by avoiding a complexity of these factors affecting the S/P ratio, noninvasive collection of saliva samples without any stimuli is considered to be one of the best methods. Furthermore, it is widely expected to use saliva samples in place of plasma samples for some pilot studies on pharmacokinetic or biopharmaceutical new drug design as well as for toxicological monitoring or as a specimen in legal medicine.

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