King Saud University

Saudi Pharmaceutical Journal

ORIGINAL ARTICLE

Comparative assessment of saliva and plasma for drug bioavailability and bioequivalence studies in humans

Nasir M. Idkaidek *

Pharmacy College, University of Petra, PO Box 961343, Amman, Jordan

Abstract *Aims:* To study the pharmacokinetics of selected drugs in plasma and saliva matrixes in healthy human volunteers, and to suggest using non-invasive saliva sampling instead of plasma as a surrogate in bioavailability and bioequivalence (BA/BE) studies.

Methods: Four different pilot BA/BE studies were done in 12–18 healthy humans. Saliva and plasma samples were collected for 3–5 half life values of metformin, tolterodine, rosuvastatin, and paracetamol after oral dosing. Saliva and plasma samples were assayed using LC-MSMS, and then pharmacokinetic parameters were calculated by non-compartmental analysis using Kinetica program. Effective intestinal permeability (P_{eff}) values were also optimized to predict the actual average plasma profile of each drug by Nelder-Mead algorithm of the Parameter Estimation module using SimCYP program.

Results: All studied drugs showed salivary excretion with strong correlation coefficients between saliva and plasma concentrations. The optimized P_{eff} ranged $1.44–68.3 \times 10^{-4}$ cm/s for the drugs under investigation. Saliva/plasma concentrations ratios ranged 0.17–1.5. Inter and intra individual variability of primary pharmacokinetic parameters in saliva matrix was either close to or higher than plasma matrix. This requires larger sample size in saliva studies for some drugs.

Conclusion: Our results suggest that there is a potential in BA/BE studies for saliva to be considered as a surrogate for plasma concentration, which goes along with drug regulations. The use of saliva instead of plasma in such studies makes them non-invasive, easy and with a lower clinical burden.

* Fax: + 962 6 5715570.
  E-mail address: nidkaidek@uop.edu.jo.

1. Introduction

Salivary excretion of some drugs has been reported previously as a good indicator for drug bioavailability, therapeutic drug monitoring, pharmacokinetics and also drug abuse. Saliva sampling offers simple, non-invasive and cheap method as

compared with plasma sampling with no contamination risk (Gorodischer and Koren, 1992; Ruiz et al., 2010). The rules of drug protein binding and membrane permeability on salivary excretion were previously investigated for several drugs, where a Salivary Excretion Classification System (SECS) was proposed as shown in Table 1 (Idkaidek and Arafat, 2012). High intestinal permeability corresponds to fraction absorption $F_a > 0.9$ and high protein binding corresponds to low fraction unbound fu of < 0.1 (Amidon et al., 1995; Sunil and Philip, 2009). According to SECS classification Class I drugs of high intestinal permeability and low protein binding, such as paracetamol, are subjected to salivary excretion. Class II drugs of low permeability and low protein binding, such as metformin, are subjected to salivary excretion since low permeability is counterbalanced by low protein binding. Class III drugs of high intestinal permeability and high protein binding, such as tolterodine, are subjected to salivary excretion since high protein binding is counterbalanced by high permeability. Class IV drugs of low intestinal permeability and high protein binding, such as montelukast, are not subjected to salivary excretion (Idkaidek and Arafat, 2012).

Four pilot studies were previously done in our laboratory on SECS class I drugs: paracetamol and tolterodine, SECS class II drug: metformin and SECS class III drug: rosuvastatin. Results were promising and have demonstrated high saliva-plasma correlations with relatively higher variability in saliva parameters (Idkaidek and Arafat, 2014a, 2014b, 2015, 2016).

Table 1 Salivary Excretion Classification System (SECS) according to drug permeability (P_{eff}) and fraction unbound to plasma proteins (fu).

Class	Parameter		
	P_{eff}	fu	Salivary excretion
Class I	High	High	Yes
Class II	Low	High	Yes
Class III	High	Low	Yes
Class IV	Low	Low	No
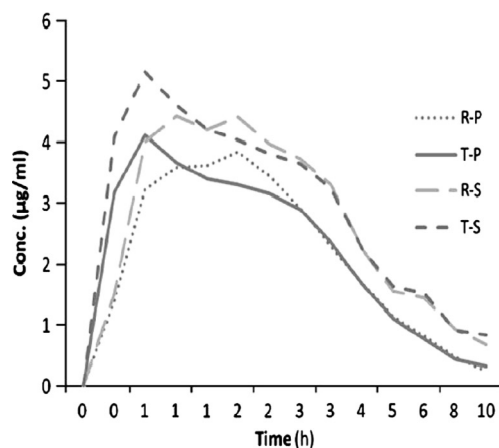
2. Objective

The objective of this review was to further investigate the robustness of using non-invasive saliva sampling method instead of plasma sampling method as a surrogate for bioavailability and bioequivalence studies of SECS classes I, II and III drugs that are excreted in saliva.

3. Experimental

Saliva BA/BE under fasted state, in 12–18 healthy human volunteers after signing informed consent, was compared to plasma pharmacokinetics in crossover or parallel design studies. Medical history, vital signs, physical examination, and laboratory safety test results showed no evidence of clinically significant deviation from normal medical condition as evaluated by the clinical investigator. The pilot bioavailability study was conducted as per the ICH, GCP, and Helsinki declaration guidelines after IRB of International Pharmaceutical Research Center and Jordan FDA approvals. Single oral doses of study drugs were administered after 10 h overnight fasting without dietary restrictions. Then resting saliva (without stimulation) and plasma samples were collected at specific time intervals up to 3–5 half lives. Thorough rinsing of the mouth was done after dosing to avoid contamination of saliva samples with any drug residues. Sensitive and accurate LC-MS/MS methods were developed and validated for the determination of study drugs in human plasma and saliva (Idkaidek and Arafat, 2014a, 2014b, 2015, 2016).

4. Data analysis

4.1. Pharmacokinetic analysis

Individual pharmacokinetic parameters for drug concentration in both saliva and plasma samples were calculated by non-compartmental analysis (NCA) using Kinetica program V5. Investigated pharmacokinetic parameters were area under the concentration curves to last collection time (AUC_t), maximum measured concentration (C_{max}) and time to maximum concentration (T_{max}).
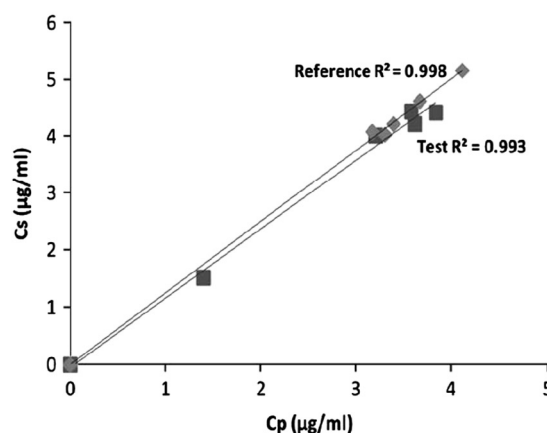

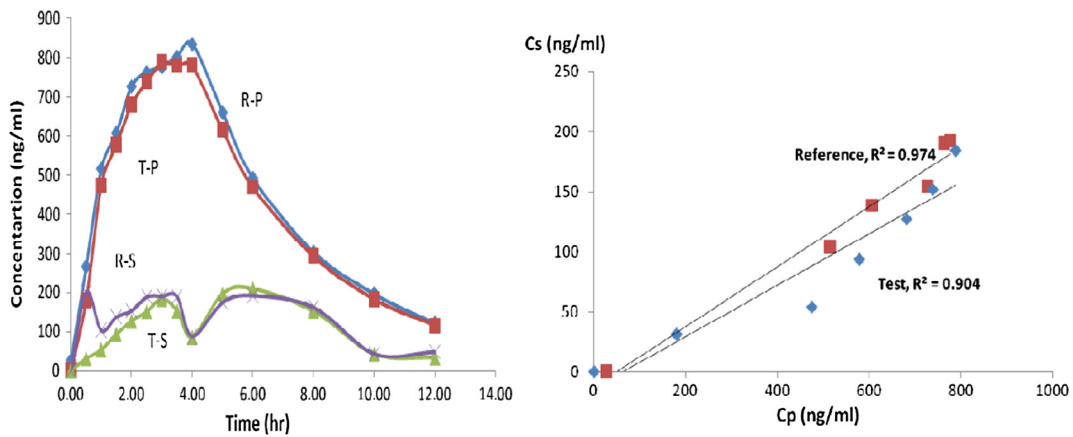**Figure 1**   Paracetamol plasma and saliva mean profiles & correlations.

Figure 2 Metformin plasma and saliva mean profiles & correlations.
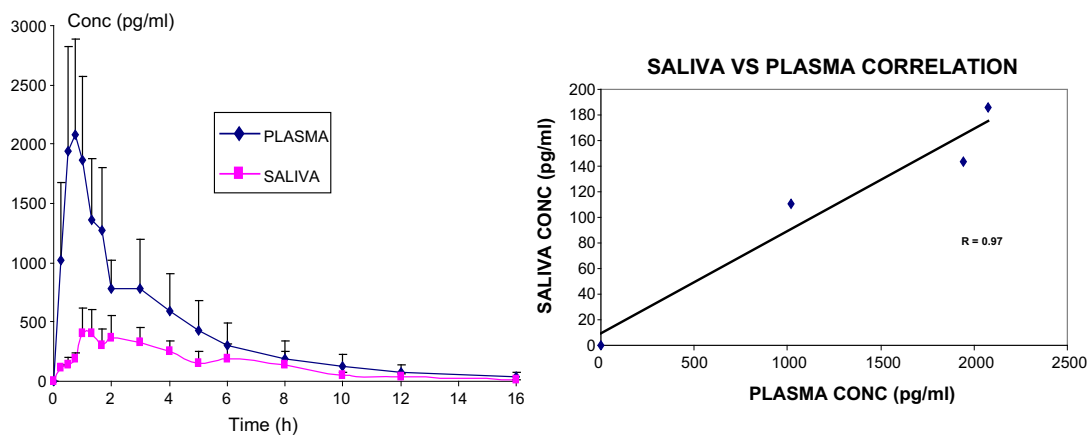

Figure 3 Tolterodine plasma and saliva mean profiles & correlations.
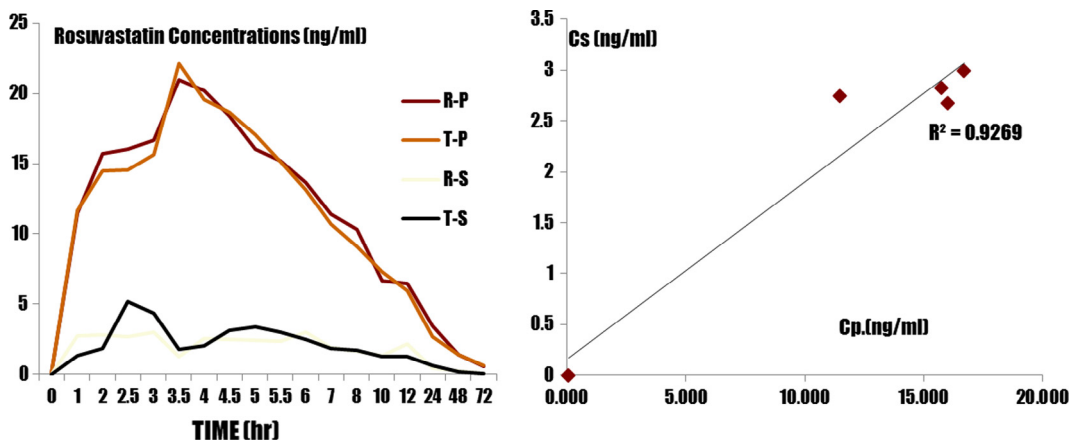

Figure 4 Rosuvastatin plasma and saliva mean profiles & correlations.

4.2. Dimensional and correlation analysis

Saliva versus plasma concentration up to median T_{max} value of plasma data was correlated by linear regression using Microsoft Excel. On the other hand, dimensional analysis was done on individual bases. This offers an advantage of more clear comparisons since ratios are unit less. The following dimensionless ratios were calculated:

$$AUC_t^* = \text{saliva } AUC_t/\text{plasma } AUC_t$$
$$T_{max}^* = \text{saliva } T_{max}/\text{plasma } T_{max}$$
$$C_{max}^* = \text{saliva } C_{max}/\text{plasma } C_{max}$$

$C^* = $ saliva Concentration/plasma concentration $= C_s/C_p$

$P^*_{eff} = $ dimensionless effective permeability $= (R \cdot P_{eff})/D$

where D is drug diffusivity as predicted by SimCYP.

4.3. Absorption kinetics

Effective intestinal permeability (P_{eff}) values were estimated by Nelder-Mead algorithm of Parameter Estimation module using SimCYP program (Jamei et al., 2009). Nelder-Mead method, which is also called downhill simplex, is a commonly used nonlinear optimization algorithm. This was done by searching for the best parameter values that produce plasma concentration that matches the actual plasma concentration at the same time. The objective function is the weighted sum of squared differences of observed and model predicted values. Polar surface area (PSA) was used first, using SimCYP, to predict initial estimate of P_{eff}. Fraction absorption (Fa) was calculated according to equations below:

$$Fa = 1 - e^{-2An}$$
$$An = P_{eff} \cdot t_{res}/R$$

where An is the absorption number; R and t_{res} are radius, set at 1.75 cm, and mean residence time, set at 3 h, in the human small intestine respectively (Takamatsu et al., 2001).

5. Results and discussion

All reviewed drugs showed good salivary excretion with strong correlation coefficient between saliva and plasma concentrations up to median T_{max} values of plasma profiles as shown in Figs. 1–4. Assuming one compartment linear model, salivary excretion rate is dependent on plasma drug concentration. This explains the close behavior of the saliva and plasma profiles. Dimensional analysis of all drugs under review is summarized in Table 2. AUC_t^* and C_{max}^* values were in close agreement with C^* values. This means that when C^* is less than unity, AUC_t^* and C_{max}^* are also less than unity as in tolterodine, metformin and rosuvastatin. On the other hand such parameters are more than unity in paracetamol.

However, T_{max}^* values were more than unity, suggesting a lag time between plasma and saliva compartments due to drug distribution/redistribution processes in the body.

On the other hand, intra/inter subject variability values for primary pharmacokinetic parameters in saliva matrix were close to or more than plasma matrix as shown in Table 3 and Table 4. The optimum sample size, as calculated by Study Result program V1, showed that more subjects are needed in pivotal studies using saliva matrix as compared to plasma matrix to demonstrate bioequivalence with adequate power of more than 80%.

This explains why 90% confidence intervals shown in Table 3 and Table 4 did not fall within 80–125% acceptance range, for all parameters with high variability values in such pilot studies. Pilot studies are not meant to show bioequivalence, but rather to compare saliva versus plasma matrices. Pivotal studies are needed to be done in future to show bioequivalence in both saliva and plasma matrices.

Mean concentration profiles of the reference product were used to estimate the effective intestinal permeability values in plasma and saliva. Fig. 3 shows observed versus SimCYP-predicted concentration profiles with correlation coefficients of 0.88 indicating good fitting of observed concentrations. Optimized effective permeability coefficients were 10.74×10^{-4} cm/s, with Fa = 1. This confirms our previous finding that effective permeability and protein binding are major key factors in salivary excretion and our previous assumption that intestinal permeability is similar to salivary mucosal permeability (Idkaidek and Arafat, 2012).

From regulatory point of view, the US FDA guidance for industry stated "The statutory definitions of BA and BE, expressed in terms of rate and extent of absorption of the active ingredient or moiety to the site of action, emphasize

Table 2 Saliva/plasma dimensional analysis.

Parameter	Tolterodine	Paracetamol	Metformin	Rosuvastatin
AUC^*	0.42	1.36	0.27	0.17
C_{max}^*	0.34	1.14	0.38	0.35
T_{max}^*	2.37	1.47	1.29	1.47
C^*	0.34	1.46	0.39	0.18
P_{eff}^*	248.39	1111.7	21.79	890.7

Table 3 BA/BE metrics and statistics in saliva matrix.

	Tolterodine[a]	Metformin	Paracetamol	Rosuvastatin
AUC_t 90%	1199 pg·h/ml	61.8–126.6	85.2–120.3	57.7–115.9
C.I. (CV%)	(82)	(47.4)	(29.9)	(47.2)
C_{max} 90%	338 pg/ml	64.1–109.8	78.3–135.1	61.3–124.8
C.I. (CV%)	(70)	(57.6)	(46.8)	(48.0)
Optimum N	55	66	48	50

[a] BA values represent calculated AUC_t and C_{max} with inter subject CV%. Optimum N was calculated assuming intra subject CV is half inter subject CV%.

Table 4 BA/BE metrics and statistics in plasma matrix.

	Tolterodine[a]	Metformin	Paracetamol	Rosuvastatin
AUC_t 90%	3152 pg·h/ml	68.8–134.9	96.8–109.1	72.7–111.4
C.I. (CV%)	(77)	(48.6)	(10.2)	(28.8)
C_{max} 90%	1237 pg/ml	69.8–125.1	86.8–116.5	76.3–151.6
C.I. (CV%)	(77)	(38.6)	(25.2)	(46.4)
Optimum N	48	54	24	48

[a] BA values represent calculated AUC_t and C_{max} with inter subject CV%. Optimum N was calculated assuming intra subject CV is half inter subject CV%.

the use of pharmacokinetic measures in an accessible biological matrix such as blood, plasma, and/or serum to indicate release of the drug substance from the drug product into the systemic circulation" (Food and Drug Administration, 2003).

Hence, from the data collected for drugs in SECS classes I, II and III there is a high potential in BA/BE studies for saliva to be considered as a surrogate for plasma concentration. This line of research can help validate the newly proposed salivary excretion classification system. The use of saliva instead of plasma in such studies makes them non-invasive, easy and with lower clinical cost, less clinical staff and less clinical burden.

More research studies of candidate drugs that fall into classes I, II and III will be done in order to compare saliva versus plasma bioavailability and bioequivalence; and demonstrate SECS robustness.

Acknowledgments

References

Amidon, G.L., Lennernäs, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12 (3), 413–420.

Food and Drug Administration, 2003. Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations. Division of Drug Information, Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane Rockville, MD 20857, USA, March.

Gorodischer, R., Koren, G., 1992. Salivary excretion of drugs in children: theoretical and practical issues in therapeutic drug monitoring. Dev. Pharmacol. Ther. 19 (4), 161–177.

Idkaidek, Nasir, Arafat, Tawfiq, 2012. Saliva versus plasma pharmacokinetics: theory and application of a salivary excretion classification system. Mol. Pharm. 9, 2358–2363.

Idkaidek, N., Arafat, T., 2014a. Saliva vs. plasma bioequivalence of paracetamol in humans: validation of class I drugs of the salivary excretion classification system. Drug Res. 64 (10), 559–562.

Idkaidek, N., Arafat, T., 2014b. Saliva vs. plasma bioequivalence of metformin in humans: validation of class II drugs of the salivary excretion classification system. Drug Res. 64 (10), 559–562.

Idkaidek, N., Arafat, T., 2015. Saliva vs. plasma bioequivalence of rosuvastatin in humans: validation of class III drugs of the salivary excretion classification system. Drugs Res. 15 (1), 79–83.

Idkaidek, N., Najib, N., Salem, II., Najib, O., 2016. Saliva vs. plasma bioavailability of tolterodine in humans: validation of class I drugs of the salivary excretion classification system. Drugs Res. 66 (6), 312–315.

Jamei, M., Turner, D., Yang, J., Neuhoff, S., Polak, S., Rostami-Hodjegan, A., Tucker, G., 2009. Population-based mechanistic prediction of oral drug absorption. AAPS J. 11 (2), 225–237.

Ruiz, M.E., Conforti, P., Fagiolino, P., Volonté, M.G., 2010. The use of saliva as a biological fluid in relative bioavailability studies: comparison and correlation with plasma results. Biopharm. Drug Dispos. 31 (8–9), 476–485.

Jambhekar, Sunil S., Breen, Philip J., 2009. Basic Pharmacokinetics. Pharmaceutical Press, London.

Takamatsu, N., Kim, O.N., Welage, L.S., Idkaidek, N.M., Hayashi, Y., Barnett, J., Yamamoto, R., Lipka, E., Lennernäs, H., Hussain, A., Lesko, L., Amidon, G.L., 2001. Human jejunal permeability of two polar drugs: cimetidine and ranitidine. Pharm. Res. 18 (6), 742–744.