Pharmacokinetics and bioavailability derived from various body fluids

Saliva samples instead of plasma samples

Willi Cawello, Schwarz BioSciences, Monheim am Rhein
Overview

- Introduction
- Sampling tissues/fluids to characterize PK
- Model of saliva sampling
- Examples
- Discussion, Conclusion / Perspectives
Definitions

- Regulatory Definition (21 CFR 320.1(a)):

  “Bioavailability means the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action.”

- Regulatory Definition (21 CFR 320.1(e)):

  “Bioequivalence means the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.”

Collected from a presentation of Kofi A. Kumi, Office of Clinical Pharmacology and Biopharmaceutics Division of Pharmaceutical Evaluation III, ACPS 7/20/2001
21 CFR 320.24

The following in vivo and in vitro approaches, in descending order of accuracy, sensitivity, and reproducibility, are acceptable for determining the bioavailability or bioequivalence of a drug product:

- Blood/plasma/serum drug conc. measurement in humans
- Urinary excretion in humans
- In vivo pharmacological effect
- Well-controlled clinical trials
- In-vitro test
- Any other approach deemed adequate by FDA

Collected from a presentation of Kofi A. Kumi, Office of Clinical Pharmacology and Biopharmaceutics Division of Pharmaceutical Evaluation III, ACPS 7/20/2001
Pharmacokinetics

- blood/plasma
- elimination
- distribution
- dose
- peripheral tissue
- movement of molecules

Pharmacodynamics

- site of action
- interaction with receptor
- stimulus
- effect
- cascade to the effect
- excretory comp.
Excretion

- excretory comp.
  - metabolism
  - renal elimination
  - salivatory comp.
Tissues
MU musculature
SA salivatory gland
SK skin
TE testis
GU thin intestine
ST stomach
SP spleen
LI liver
PA pancreas
LU lung
HT heart
BR brain
AD fat tissue
KI kidney
Pharmacokinetics in saliva

Mass of saliva glands: 30-50g
Approximated Blood flow: 50-200mL/min
Pharmacokinetics in saliva

What about mass balance during extraction to saliva?

Having a 1:1 extraction:
Concentration in plasma and in saliva is the same.

**Plasma**
Before ‘extraction’ 5µg/mL
After ‘extraction’ 4.902µg/mL\(\frac{5 \times 50}{50+1}\)

**Saliva**
Concentration 4.902µg/mL \(\frac{5 \times 50}{50+1}\)

Total Amount in Plasma (central circulation)
Before: 12.5mg \((2500 \text{ml} \times 5\mu g/\text{mL})\)
After: 12.495mg \(= 4.998\mu g/\text{mL}\)
Prediction of extraction into saliva

Ratio of saliva vs. plasma concentrations

\[ R = \frac{1 + 10^{pH_{\text{saliva}} - pKa}}{1 + 10^{pH_{\text{plasma}} - pKa}} \cdot \frac{f_{u,\text{plasma}}}{f_{u,\text{saliva}}} \]

- \( pH_{\text{plasma}} \) is almost constant,
- \( f_u = \text{fraction unbound} \), \( f_{u,\text{saliva}} \) is 1 for most drugs,
- \( pKa \) is the negative logarithm of acid dissociation constant

Equation derived from:
### Elimination by saliva

#### Examples of drug elimination by saliva (ratios collected from the literature)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ratio of saliva conc vs plasma conc</th>
<th>Protein binding %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminophenazon</td>
<td>0.79</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Amphetamin</td>
<td>2.76</td>
<td></td>
</tr>
<tr>
<td>Antipyrin</td>
<td>0.89 - 1.00</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>0.26 - 0.44</td>
<td>75</td>
</tr>
<tr>
<td>Coffein</td>
<td>0.55</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0.029</td>
<td>95-98</td>
</tr>
<tr>
<td>Digoxin</td>
<td>0.66 - 1.68</td>
<td>20-30</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>1.02</td>
<td>10</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>1.3</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Lithium</td>
<td>2.86 - 3.43</td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>1.40</td>
<td>20-50</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.015</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>0.60</td>
<td>35</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.30 - 0.43</td>
<td>59</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>0.09 - 0.24</td>
<td>83-94</td>
</tr>
<tr>
<td>Sulfanilamid</td>
<td>0.87 - 1.08</td>
<td>20</td>
</tr>
<tr>
<td>Theophyllin</td>
<td>0.49 - 0.77</td>
<td>56</td>
</tr>
</tbody>
</table>
Examples of saliva sampling for PK evaluation

Study of bioavailability and pharmacokinetics of theophylline following administration of two sustained release dosage forms as assessed by salivary data: Part II

N. Ohmori, N. Inotsume, M. Matsukura, A. Higashi, R. Iwaoku, Y. Tobino, M. Nakano and I. Matsuda

1Department of Pharmaceutical Services, Kumamoto University Hospital and 2Department of Pediatrics, Kumamoto University Medical School, 1-1-1 Honjo, Kumamoto 860, Japan

Abstract. Bioavailability and pharmacokinetics of theophylline following administration of a marketed sustained release tablet (Theo-Dur®) and a newly designed sustained release tablet (E-0686) have been studied in fifteen healthy volunteers by measuring plasma and salivary concentrations. The theophylline level in saliva was 42.3 ± 0.008 (s.e.m.) % of the plasma level and its correlation coefficient was 0.908. This observation suggests that salivary levels are considered to be a good indicator of the plasma concentration of theophylline. Inter-

International Journal of Clinical Pharmacology, Therapy and Toxicology, Vol. 24 No. 4 – 1986 (pp. 196–201)
Examples of saliva sampling for PK evaluation

\[
\text{Conc}_{\text{saliva}} \approx 0.423 \times \text{Conc}_{\text{plasma}}, \quad r=0.908
\]

*Fig. 1* Relationship between theophylline concentrations in 600 plasma and salivary specimens.

Examples of saliva sampling for PK evaluation

Fig. 2  Mean plasma (●) and salivary (▲) theophylline concentrations after administration of 300 mg Theo-Dur under fasting (----) and non-fasting (-----) conditions in 15 healthy subjects.

Fig. 3  Mean plasma (○) and salivary (△) theophylline concentrations after administration of 300 mg E-0686 under fasting (----) and non-fasting (-----) conditions in 15 healthy subjects.

Examples of saliva sampling for PK evaluation

\[ \text{conc}_{\text{saliva}} = -0.51 \pm 0.71 + 1.316 \pm 0.066 \times \text{conc}_{\text{plasma}} \]

Figure 3 Levetiracetam saliva vs. plasma concentrations relationship. The line represents the least-squares regression line.

Published by: Fontain N.B., et al, Epilepsy Research 2007, in press
Examples of saliva sampling for PK evaluation

Concentrations after single oral dose of 750mg Levetiracetam (three 250mg tablets)
Examples of saliva sampling for PK evaluation

Example: drug RED
Comparison of plasma PK and saliva PK

Concentrations of RED

\[ y = 1.1484x - 0.0333 \]

\[ R^2 = 0.8775 \]
Comparison of plasma PK and saliva PK

Concentrations of RED metabolite

\[ y = 1.0515x + 0.0062 \]

\[ R^2 = 0.8676 \]
**Parameters of pharmacokinetics evaluation of bioequivalence**

<table>
<thead>
<tr>
<th>Treatment drug</th>
<th>AUC Plasma</th>
<th>AUC Saliva</th>
<th>BE % plasma</th>
<th>BE % saliva</th>
<th>90%CI plasma</th>
<th>90%CI saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td>A RED</td>
<td>86.4/18.3</td>
<td>95.3/20.3</td>
<td>101.4</td>
<td>99.9</td>
<td>89.0-115.5</td>
<td>88.1-113.3</td>
</tr>
<tr>
<td>B RED</td>
<td>85.1/17.6</td>
<td>94.9/18.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A metabolite</td>
<td>10.05/4.11</td>
<td>11.02/4.82</td>
<td>101.4</td>
<td>102.6</td>
<td>75.7-134.9</td>
<td>74.1-142.1</td>
</tr>
<tr>
<td>B metabolite</td>
<td>9.98/4.11</td>
<td>10.73/4.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Arithmetic mean/sd, n=16, BE and 90% CI after log transformation
Model dependent PK with plasma or saliva samples

PK evaluation of data set 6 (method=monquardt, weight=1/pp)
One compartment model with first order absorption and first order elimination (Bateman function with parameters $k_a$, $k_e$, dose, and $V_d$)

<table>
<thead>
<tr>
<th>Saliva samples</th>
<th>Plasma samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_e = 0.0622 \text{ h}^{-1}$</td>
<td>$k_e = 0.0635 \text{ h}^{-1}$</td>
</tr>
<tr>
<td>$t_{1/2} = 11 \text{ h}$</td>
<td>$t_{1/2} = 11 \text{ h}$</td>
</tr>
<tr>
<td>$t_{\text{lag}} = 0.245 \text{ h}$</td>
<td>$t_{\text{lag}} = 0.187 \text{ h}$</td>
</tr>
<tr>
<td>$V/f = 42.2 \text{ L}$</td>
<td>$V/f = 49.6 \text{ L}$</td>
</tr>
</tbody>
</table>
Comparison of plasma PK and saliva PK

- Data from saliva samples are sufficient to characterize PK profile, model dependent PK and useful for BE evaluation.

- Rate constant of elimination resp. $t_{1/2}$ is the same.

- Approximation of the volume of distribution using saliva samples results in about 15% lower values corresponding to 15% higher concentrations in saliva than in plasma.

- Similar results for the metabolite of RED.

Pharmacokinetics of RED in saliva is a good approximation (a surrogate) for the pharmacokinetics in plasma.
Examples of saliva sampling for PK evaluation

Example: drug BLUE
Correlation between BLUE concentrations in saliva and plasma

\[ y = 0.681x - 0.0038 \]

\[ R = 0.7811 \]
Correlation between BLUE concentrations in saliva and plasma

Individual factor for correlation between plasma and saliva concentration

\[ C_{\text{saliva}} = \text{intercept} + \text{factor} \times C_{\text{plasma}} \]

<table>
<thead>
<tr>
<th>parameter</th>
<th>intercept</th>
<th>factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>arithmetic mean</td>
<td>-0.018</td>
<td>0.671</td>
</tr>
<tr>
<td>SD</td>
<td>0.242</td>
<td>0.273</td>
</tr>
<tr>
<td>range</td>
<td>-1.04 - 0.586</td>
<td>0.297 – 1.424</td>
</tr>
</tbody>
</table>
Correlation between BLUE concentrations in saliva and plasma (after individual correction)

\[ y = 0.9903x + 0.0488 \]

\[ R = 0.8687 \]
Results of „BE“ evaluation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Medium</th>
<th>8mg BLUE - reference</th>
<th>8mg BLUE - test</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$</td>
<td>Plasma, Saliva</td>
<td>3.69(45.1), 3.17(63.5)</td>
<td>4.16(43.7), 3.47(65.2)</td>
</tr>
<tr>
<td>$\text{AUC}_{(0-tz)}$</td>
<td>Plasma, Saliva</td>
<td>44.9(43.8), 33.3(65.2)</td>
<td>47.5(41.5), 34.4(63.0)</td>
</tr>
<tr>
<td>$t_{\text{max}}$</td>
<td>Plasma, Saliva</td>
<td>5(3-6), 5(3-8)</td>
<td>5(3-6), 5(3-8)</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$ and $\text{AUC}_{(0-tz)}$: Mean (CV), $t_{\text{max}}$: range
## Results of „BE“ evaluation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saliva</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>point estimate</td>
<td>90% CI</td>
<td>point estimate</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>112.0</td>
<td>98.4 – 127.5</td>
<td>112.3</td>
</tr>
<tr>
<td>$\text{AUC(0-tz)}$</td>
<td>105.7</td>
<td>94.9 – 117.8</td>
<td>106.6</td>
</tr>
</tbody>
</table>

ANOVA results relative bioavailability
PK modelling

plasma

concentration [ng/mL]

0.1 1.0 10.0

time [h]

0 6 12 18 24 30 36

saliva

concentration [ng/mL]

0.1 1.0 10.0

time [h]

0 6 12 18 24 30 36
PK modelling

PK model: one compartment model with first order absorption and elimination (Bateman function)

\[ k_e \text{ (1/h)} \]

<table>
<thead>
<tr>
<th></th>
<th>treatment A (n=33)</th>
<th>treatment B (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>plasma</td>
<td>0.1548 +/- 0.2084</td>
<td>0.1357 +/- 0.0596</td>
</tr>
<tr>
<td>saliva</td>
<td>0.1319 +/- 0.0792</td>
<td>0.1309 +/- 0.0595</td>
</tr>
</tbody>
</table>
Comparison of plasma PK and saliva PK

• Saliva samples are a good surrogate for model independent PK and PK modelling (for drug BLUE in plasma).

• BE evaluation based on the saliva samples fit with the results of the evaluation based on plasma samples (especially after normalization using individual factors).

Use saliva samples instead of plasma samples for characterization of BLUE PK (after validation of the correlation and characterization of variability and maybe use of individual value of R estimated with 1 or 2 plasma samples).
Discussion and Conclusion

- Using saliva samples instead of / in addition to plasma samples was shown to be a feasible tool to characterize the PK of a drug in plasma.

- Using a drug specific ratio for saliva over plasma concentrations can result in useful values for model dependent and model independent parameters of PK and BE testing.

- The BE evaluation based on saliva samples fits with the results based on plasma samples (especially after normalization using individual factors).
Future perspectives

- Saliva samples not only for therapeutic drug monitoring but for PK / popPK evaluation
- Decreased costs for sample collection
- Increased explanatory power of Population PK with possible (more) sampling out of clinics (at more relevant time points after admin.)
- Increased acceptance for studies in children (by patients, parents, ethics committees, and authorities)
- Increased comfort for the patients/subjects by non-invasive sampling

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