Optimizing levofloxacin dose in the treatment of multidrug-resistant tuberculosis

Ghimire, Samiksha

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 15-06-2020
Evaluation of saliva as a potential alternative sampling matrix for therapeutic drug monitoring of levofloxacin in MDR-TB patients

Samiksha Ghimire, Bhagwan Maharjan, Erwin M. Jongedijk, Jos G.W. Kosterink, Gokarna R. Ghimire, Daan J. Touw, Tjip S. van der Werf, Bhabana Shrestha, Jan-Willem C. Alffenaar

Antimicrobial Agents and Chemotherapy. 2019 Feb; 63 (5): 02379–18
ABSTRACT

Saliva may be a useful alternative matrix for monitoring levofloxacin concentrations in multi-drug resistant TB patients. The objectives of this study were: a) to evaluate the correlation between plasma and salivary Lfx concentrations in MDR-TB patients; and b) to gauge the possibility of using saliva as an alternative sampling matrix for therapeutic drug monitoring of Lfx in TB endemic areas. This was a prospective pharmacokinetic study that enrolled MDR-TB patients receiving levofloxacin (Lfx; 750–1000 mg once daily dosing) under standardized treatment regimen in Nepal. Paired blood and saliva samples were collected at steady state. Lfx concentrations were quantified using liquid chromatography-tandem mass spectrometry. Pharmacokinetic parameters were calculated using non-compartmental kinetics. Lfx drug exposure was evaluated in 23 MDR-TB patients. During the first month, the median (IQR) area under the concentration-time curve (AUC\textsubscript{0–24}) was 67.09 (53.93–98.37) mg*h/L in saliva and 99.91 (76.80–129.70) mg*h/L in plasma, and the saliva plasma (S/P) ratio was 0.69 (0.53–0.99). Similarly, during the second month, the median (IQR) AUC\textsubscript{0–24} was 75.63 (61.45–125.5) mg*h/L in saliva and 102.7 (84.46–131.9) mg*h/L in plasma with a S/P ratio of 0.73 (0.66–1.18). Furthermore, large inter- and intra-individual variabilities in Lfx concentrations were observed. This study could not demonstrate a strong correlation between plasma and saliva Lfx levels. Despite a good Lfx penetration in saliva, the variability in individual saliva-to-plasma ratios limits the use of saliva as a valid substitute for plasma. Nevertheless, saliva could be useful in semi-quantitatively predicting Lfx plasma levels.
INTRODUCTION

Levofloxacin (Lfx) belongs to the group A fluoroquinolones (FQ) for treating multi-drug resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid and rifampicin (1). This class of drug is used throughout the course of treatment in the new, shorter nine-month regimen, in the longer 24-month MDR-TB regimen and additionally in the six-month regimen for rifampicin susceptible, isoniazid monoresistant TB (2). Lfx and moxifloxacin have been used interchangeably in the longer regimen, however, in developing countries Lfx is preferred due to affordability, availability, better safety profile and fewer drug interactions with other medications (3,4). Acquired FQs resistance during standard treatment resulting in poor outcomes shown in a prospective observational cohort study is of serious concern (5). An earlier study by the same group showed that 11.2 % (79/832) of MDR-TB patients developed FQ resistance without any baseline resistance, potentially due to sub-therapeutic systemic concentrations of drugs achieved (6, 7). Similarly, other pharmacokinetic studies on Lfx in MDR-TB patients found considerable pharmacokinetic variability among individuals, with at least 25 % of the patients not attaining desired plasma concentration and area under the concentration vs time curve (AUC$_{0–24}$) (3, 4, 8, 9). It is clear that Lfx concentrations do not always reach the desired concentrations while administered in a standard dose. Therefore, measuring Lfx concentrations in plasma or other alternative matrices (saliva and dried blood spots) could help clinicians make informed dosing decisions. More so now, as the TB treatment marches towards individualization, therapeutic drug monitoring (TDM) using saliva sampling might become a game changer in TB treatment due to specific advantages over plasma sampling, in low resource settings (10, 11). The efficacy of Lfx is predicted by AUC$_{0–24}$ and minimum inhibitory concentration (MIC) (12). Given as a monotherapy, the hollow fiber infection model on tuberculosis recently established a Lfx target of 146 for maximum bacterial kill (EC$_{80}$) and 360 for the prevention of acquired drug resistance (12). Therefore, plasma AUC$_{0–24}$ above 75 (if MIC is 0.5 mg/L) or 146 (if MIC is 1 mg/L) will be needed to attain the optimal target exposure for efficacy. With standard 750–1000 mg once daily dose, desired median peak concentration ($C_{\text{max}}$) was 8–13 mg/L.
while, median time to reach $C_{\text{max}}$ ($t_{\text{max}}$) was 1–2 h and median half-life ($t_{1/2}$) was 6–8 h (13–15). Lfx demonstrated good penetration in extravascular body sites such as cerebrospinal fluid and cavitary lesions, due to rapid absorption and high volume of distribution (16, 17). Sasaki and Morishima compared Lfx levels in saliva and serum of eight healthy male volunteers after administration of 100 mg single dose. The study reported mean saliva/serum Lfx AUC ratio of 0.69 in fasting state and 0.56 in non-fasting state, indicating that saliva Lfx concentration could be useful for TDM (18). To date, however, studies comparing Lfx concentrations in plasma and saliva of MDR-TB patients have not been published. Saliva could be a useful alternative in predicting Lfx concentrations from plasma since sampling is non-invasive, fast, requires less rigid storage conditions, can be easily transported and is more suitable in children (19).

Therefore, the aims of this study were as follows: a) to evaluate the correlation between plasma and salivary Lfx concentrations in MDR-TB patients; and b) to gauge the possibility of using saliva as an alternative sampling matrix for TDM of Lfx in TB endemic areas.

**PATIENTS AND METHODS**

**Patients and design**

Study participants were MDR-TB patients undergoing treatment at German Nepal Tuberculosis Project (GENETUP), Nepal. This was a prospective pharmacokinetic study that enrolled patients on treatment during 25/05/2016–27/10/2017, with signed informed consent. The study protocol was approved by Ethical Review Board of Nepal Health Research Council, Kathmandu, Nepal (Reg. No 115/2016) and registered at clinicaltrials.gov (identifier number NCT 03000517). Patients (≥18 years) with newly diagnosed or previously treated MDR-TB (based on genotypic susceptibility testing to rifampicin by GeneXpert and culture) receiving Lfx as a part of their MDR-TB regimen were eligible for inclusion. Subjects were excluded if they had neurologic or severe extra-pulmonary manifestations of TB; had a body weight <35 kg, were on medications for the treatment of renal disorders, were breast feeding or pregnant, were treated with aluminum- and magnesium containing
antacids or ferrous sulphate, cimetidine, probenecid, theophylline, warfarin, zidovudine, digoxin or cyclosporine due to potential drug-drug interactions.

The national tuberculosis guidelines for the programmatic management of MDR-TB in Nepal consists of an intensive phase of 8 months (with an addition of 4 months if there is no culture/ conversion at the end of 6 months) followed by a continuation phase of 12 months of treatment. Lfx (750–1000 mg once daily) was prescribed based on body weight as described in the guidelines for management of drug resistant tuberculosis in Nepal. Other drugs in this regimen included kanamycin (500–1000 mg/day i.m. injection), ethionamide (500–750 mg/day), pyrazinamide (20–30 mg/kg/day) and cycloserine (500–750 mg/day).

**Study procedures**

To assess Lfx concentrations, steady state blood and saliva samples were collected before intake and at 1, 2, 4 and 8 hours after intake of Lfx. Patients were sampled twice i.e. at the end of the first month (15–30th day) and second month (45–60th day) of treatment. Plasma samples were collected in BD vacutainer vials (Becton, Dickinson and Company, NJ, USA, catalog no. 23–021–016) whereas, saliva samples were collected using two different techniques. Saliva samples were collected using a salivette® (Sarstedt, Nümbrecht, Germany, catalog no. 50-809-199) and additionally filtered using a membrane filter (0.2 µm diameter, Merck KGaA, Darmstadt, Germany)(20). The collected plasma/saliva samples were centrifuged and frozen at −30 °C until analysis. A standardized data collection (case report forms and excel database file) was created to record demographic and clinical data of the included patients. HIV test was carried out for all included patients as a part of treatment protocol, but none of the included patients were HIV positive.

**Drug quantification in plasma and saliva**

Lfx concentrations in human plasma and saliva were analyzed in the laboratory of the department of Clinical Pharmacy and Pharmacology at the University Medical Center Groningen, Netherlands using a validated liquid-chromatography tandem mass spectrometry technique (LC-MS/MS)(21). The calibration curve was linear over a range of 0.10–5 mg/L for Lfx. To encompass concentrations levels above 5 mg/L,
dilution integrity was determined to accurately measure concentrations levels up to 40 mg/L.

The pH of salivary samples was measured using a pH indicator strips (Merck KGaA, Darmstadt, Germany), encompassing the pH range from 2.0–9.0, with 0.5 pH units increment distinguished by color change. Two independent observers (S.G., SHJ.VDE.) recorded the results, and in case of differences consensus was reached in the presence of a third observer (A.GM.).

Data analysis
PK analysis. For PK parameters, non-compartmental analysis was performed using MW/Pharm (version 3.82; Mediware, Groningen, the Netherlands). The PK parameters included: maximal plasma concentration (C_{\text{max}}), corresponding time of C_{\text{max}} (t_{\text{max}}), area under plasma concentration vs. time curve (AUC_{0–24}), apparent volume of distribution (Vd/F), apparent clearance (CL/F), half-life (t_{1/2}) and elimination constant for plasma and saliva (ke). Statistical analysis was performed using SPSS Inc. (v 23.0, Chicago IL, USA). Results are presented as medians with interquartile range (IQR) for continuous variables and number percentage (%) for categorical variables. The normal distribution of data was ascertained by skewness-kurtosis, visual inspection of boxplots and Shapiro-Wilk test. The non-parametric Wilcoxon signed rank test was used to assess the differences between plasma and saliva PK parameters, when applicable. Inter- and intra- individual pharmacokinetic variabilities were evaluated from the CV% calculated as the quotient of standard deviation divided by the mean plasma concentration multiplied by 100. Passing-Bablok regression was used to assess the relationship between saliva and plasma Lfx concentrations (R Statistical Software). All P values were reported as significant if P <0.05.

RESULTS

Study subjects. Twenty-three MDR-TB patients were included in the study and demographic and baseline clinical characteristics are shown in Table 1. In our study, 70% (16/23) were male. The median age was 32 (IQR 28–47 years), body weight was 48 (IQR 41–55 kg) with
a body-mass index (BMI) of 18 (IQR 16–19 kg/m$^2$). Based on BMI, 65% (15/23) of the patients were underweight, as a result once daily 750–1000 mg Lfx dosing resulted in mg/kg doses of 17.14 (15.38–19.23). All 23 (100%) patients completed the first PK sampling (15–30th day). However, during the second month, 4 (13.1%) patients failed to participate in PK sampling. One patient was transferred out, while two patients were shifted to pre-XDR category, whereas the remaining patient participated only in saliva sampling.

**Lfx PK.** The steady-state Lfx PK parameters are mentioned in Table 2. During the first month, the median area under the concentration-time curve (AUC$_{0-24}$) was 67.09 (IQR 53.93–98.37) mg*h/L in saliva and

<table>
<thead>
<tr>
<th>Table 1: Baseline characteristics of all included patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Characteristics</td>
</tr>
<tr>
<td>Demographic (n=23)</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Body weight, kg</td>
</tr>
<tr>
<td>Length, cm</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
</tr>
<tr>
<td>Underweight (&lt;18.5 kg/m$^2$)</td>
</tr>
<tr>
<td>Normal (18.5–25.0 kg/m$^2$)</td>
</tr>
<tr>
<td>Co-morbidities</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>HIV</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
</tr>
<tr>
<td>Lfx</td>
</tr>
<tr>
<td>Renal function, baseline</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
</tr>
<tr>
<td>Urea, mg/dl</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
</tr>
</tbody>
</table>

Data are presented as n (%) or median (interquartile range)
Table 2: Steady-state pharmacokinetic parameters for Lfx

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plasma</th>
<th>Saliva</th>
<th>S/P ratio</th>
<th>S/P ratio</th>
<th>p-value (plasma)</th>
<th>p-value (saliva)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>month</td>
<td>I</td>
<td>II</td>
<td>I</td>
<td>II</td>
<td>I and II</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=23)</td>
<td>(n=19)</td>
<td>(n=23)</td>
<td>(n=20)</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–24&lt;/sub&gt;, mg*h/L</td>
<td>99.91 (76.80–129.70)</td>
<td>102.7 (84.46–131.90)</td>
<td>67.09 (53.93–98.37)</td>
<td>75.63 (61.45–125.5)</td>
<td>0.69 (0.53–0.99)</td>
<td>0.74 (0.59–0.93)</td>
</tr>
<tr>
<td>fAUC&lt;sub&gt;0–24&lt;/sub&gt;, mg*h/L</td>
<td>75.93 (58.37–98.57)</td>
<td>78.05 (64.19–100.24)</td>
<td>-</td>
<td>-</td>
<td>0.88 (0.92–0.99)</td>
<td>0.96 (0.95–1.25)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, mg/L</td>
<td>10.35 (9.10–11.44)</td>
<td>10.96 (9.34–11.58)</td>
<td>7.03 (5.61–9.02)</td>
<td>8.30 (6.56–12.03)</td>
<td>0.68 (0.53–0.97)</td>
<td>0.73 (0.66–1.18)</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt;, h</td>
<td>2 (1.08–4)</td>
<td>2 (1.02–4.06)</td>
<td>2 (1.3–4)</td>
<td>2 (1.04–3.36)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CL/F, L/h</td>
<td>6.75 (4.72–9.46)</td>
<td>7.94 (5.09–9.34)</td>
<td>9.58 (6.74–12.33)</td>
<td>8.99 (5.92–11.80)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vd/F, L</td>
<td>87.9 (72.54–106.40)</td>
<td>88.84 (55.73–101.2)</td>
<td>124.3 (111.45–157.30)</td>
<td>125.60 (83.04–158.25)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2e&lt;/sub&gt;</td>
<td>8.77 (6.50–10.71)</td>
<td>7.86 (6.32–10.11)</td>
<td>8.58 (7.97–10.36)</td>
<td>8.47 (6.23–14.02)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>k (/h)</td>
<td>0.08 (0.06–0.11)</td>
<td>0.08 (0.07–0.08)</td>
<td>0.10 (0.07–0.12)</td>
<td>0.08 (0.05–0.11)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range). AUC<sub>0–24</sub>, area under the concentration-time curve; fAUC<sub>0–24</sub>, free Lfx AUC<sub>0–24</sub> assuming plasma protein binding of 24%; C<sub>max</sub>, maximum concentration of drug; t<sub>max</sub>, time at which C<sub>max</sub> occurred; CL/F, apparent total body clearance; Vd/F, apparent volume of distribution; t<sub>1/2e</sub>, elimination half-life; k, elimination rate constant.
99.91 (IQR 76.80–129.70) mg*h/L in plasma, and the saliva plasma (S/P) ratio was 0.69 (IQR 0.53–0.99). Moreover, the median C_{max} was 7.03 (IQR 5.61–9.02) mg/L in saliva and 10.35 (9.10–11.44) mg/L in plasma with the S/P ratio of 0.68 (IQR 0.53–0.97). A moderate positive correlation (r_s=0.50; p=0.016) was demonstrated between the saliva and plasma AUC_{0–24}. Similarly, during the second month, the median AUC_{0–24} and C_{max} were 75.63 (IQR 61.45–125.5) mg*h/L and 8.30 (IQR 6.56–12.03) mg/L in saliva and 102.7 (IQR 84.46–131.9) mg*h/L and 10.96 (IQR 9.34–11.58) mg/L in plasma. The median AUC_{0–24} S/P ratio was 0.734 (IQR 0.66–1.18). This time, saliva and plasma AUC_{0–24} showed a strong positive linear relationship (r_s=0.754; p=0.0001) compared to the first month. Assuming a Lfx plasma protein binding of 24%, the median free plasma fAUC_{0–24} was 75.93 (58.37–98.57 IQR) mg*h/L in the first month and 78.05 (64.19–100.24 IQR) mg*h/L in the second month of treatment. The median S/P ratios were 0.96 (0.95–1.25 IQR) and 0.88 (0.92–0.99 IQR) respectively. The unbound Lfx fAUC_{0–24} in plasma reflected its salivary AUC_{0–24} closely, with S/P ratio almost close to 1. Lfx concentration-time curves for both plasma and saliva are shown in Figure 1.

Passing Bablok regression analysis was used to evaluate the agreement between plasma and saliva Lfx concentrations. Figure 2 shows fitted Passing-Bablok regression that revealed a linear relationship
Figure 2: Passing-Bablok regression analysis of mean Lfx concentrations (t0, 1, 2, 4, 8 h) in plasma and saliva for two months.

The bold solid line represents the Passing-Bablok fitted line, whereas the solid line is the line of identity. The dashed lines are 95% CI; r is the spearman's rank correlation; and N is the number of paired mean plasma and saliva concentrations.
and was close to the line of identity (x=y) with an estimated slope 95% CI of 1 (−2.11 to 2.57) for first month and 1.81 (−0.51 to 3.92) for second month. Similarly, the intercept was −1.85 (−9.81 to 16.42) and −7.17 (−21.26 to 0.95) respectively. In both months, 95% CI range included 1 for slope and 0 value for intercept, thereby satisfying the condition for line of identity.

The inter-individual variability was assessed in 208 Lfx measurements in plasma and 195 measurements in saliva at 0, 1, 2, 4, and 8 h samples. We found large inter-individual variability in Lfx concentrations. Furthermore, intra-individual variability was evaluated for the same patient based on the Lfx concentrations in plasma and saliva, between first and second months of treatment. The median intra-individual variability CV_{intra} was 8.77 (IQR 3.56–24.90 %) in plasma and 24.25 (IQR 12.20–34.65 %) in saliva for (19/23) patients. In our study, the intra-individual variability was lower than inter-individual variability. Table 3 shows inter-and intra-individual coefficients of variation for Lfx. The salivary pH ranged from 4.5–8.0 for different individuals with a mean value of 5.78 in the first month and 5.96 in the second month. Lfx saliva-plasma ratio as a function of salivary pH are plotted together (Figure 3). Furthermore, a trend towards moderately positive correlation (r_s=0.379; p=0.021) was observed when Lfx C_{min} in saliva was evaluated to predict its AUC_{0–24} in plasma (r= 0.38; estimated linear regression equation). When saliva C_{min} was below 2 mg/L, proportion of patients had plasma AUC_{0–24} below desired 146 (12) given MIC was at 1 mg/L.

**DISCUSSION**

The presence of Lfx in MDR-TB regimen has been associated with greater treatment success and reduced death(22). Despite this dominant position as a 2nd line TB drug, many clinical trials have shown inadequate Lfx concentrations in plasma of MDR-TB patients that has refrained the drug from achieving its maximum efficacy (4, 8, 9). The measurement of drug concentrations in plasma of MDR-TB patients and dose adjustments thereafter have contributed positively to MDR-TB treatment outcomes (23). Yet, only few TB treatment centers worldwide have adopted TDM. Officially, the importance of TDM in
Evaluation of saliva as an alternative sampling matrix for TDM of levofloxacin

The management of patient’s sub-groups of drug-susceptible tuberculosis was first introduced in the clinical practical guidelines by the American Thoracic Society, Centers for Disease Control and Prevention and, Infectious Diseases Society of America and was endorsed by the European Respiratory Society and the US National Tuberculosis Controllers association(24). Among many logistic and financial challenges

Table 3: Inter-individual (CVinter) and intra-individual (CVintra) variabilities of Lfx

<table>
<thead>
<tr>
<th>Inter-individual variability (n=23)</th>
<th>Plasma concentration, mean (SD); (CVinter%)</th>
<th>Saliva concentration, mean (SD);(CVinter%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (h)</td>
<td>I month</td>
<td>II month</td>
</tr>
<tr>
<td>0</td>
<td>1.70, 1.14; 67.29</td>
<td>1.63, 1.06; 65.39</td>
</tr>
<tr>
<td>1</td>
<td>8.26, 2.47; 41.98</td>
<td>7.23, 3.65; 50.43</td>
</tr>
<tr>
<td>2</td>
<td>8.42, 2.36; 27.98</td>
<td>9.91, 1.90; 19.13</td>
</tr>
<tr>
<td>4</td>
<td>7.61, 2.04; 25.85</td>
<td>7.84, 1.92; 24.43</td>
</tr>
<tr>
<td>8</td>
<td>6.40, 3.72; 58.11</td>
<td>6.14, 3.37; 54.89</td>
</tr>
</tbody>
</table>

Intra-Individual variability, 8.77 (3.65–24.90) * (n=19)

CVintra % 24.25 (12.20–34.65) * (n=20)

*= Median (interquartile range). SD, standard deviation; CV%, co-efficient of variation.

Figure 3: Lfx saliva-plasma ratios at different time-points (0, 1, 2, 4, 8 h) and salivary pH at first and second month of treatment.

Figure 4: Lfx C min in saliva to predict plasma AUC 0-24. The vertical line at 2 mg/L is the C min cut-off.
that have hindered TDM implementation, one problem is that venous sampling does not always have enough leverage in low-resource TB endemic settings, mainly due to the invasive nature of sampling, need of skilled personnel for venipuncture, potential infectious hazard, cooling requirements for transportation and storage, and high costs (11). In this scenario, use of alternative and stress-free sampling matrixes such as saliva could imprint TDM strategy in the national TB treatment guidelines. Therefore, in this first study on salivary penetration of Lfx in MDR-TB patients, we evaluated saliva’s potential as an alternative sampling matrix and to explore whether it can quantitatively reflect plasma concentrations for TDM guided dosing. Overall, the salivary and plasma concentration-time profiles agreed well for different patients characterized by higher Lfx concentrations in plasma than in saliva except in 21 % (5/23) of patients who had higher salivary concentrations. The amount of Lfx present in saliva is representative of its free fraction in plasma that is able to passively diffuse to saliva, which happens almost instantaneously due to a concentration gradient(25). Given Lfx’s variable degree of protein binding (24–40 %) in different individuals, we found large inter-individual variation in salivary concentrations(22). The results obtained from plasma samples were more homogenous and consistent with recently published studies on MDR-TB patients by van’t Boveneind-Vrubleuskaya et al. and Peloquin et al.

Figure 3: Lfx C\text{\textsubscript{min}} in saliva - plasma ratios at different time-points (0, 1, 2, 4, 8h) and salivary pH at first and second month of treatment.

Figure 4: Lfx C\text{\textsubscript{min}} in saliva to predict plasma AUC\textsubscript{0–24}. The vertical line at 2 mg/L is the C\text{\textsubscript{min}} cut-off.
with similar median observed AUC\textsubscript{0–24} and C\textsubscript{max} values\cite{4, 15}. In theory, several factors could explain the high inter-individual variability in saliva, such as salivary pH in combination with drug pKa, salivary flow rate, and mechanism of drug transport (passive or active)\cite{25, 26}. The degree of ionization in different compartments is generally explained by pH of the compartments and the pKa of the drug. For example, lipid soluble non-ionized drugs which are not extensively bound to plasma proteins can easily transfer across the phospholipid bilayer of salivary cell membranes compared to ionized hydrophilic ones which tend to retain in plasma \cite{26, 27}. The pKa values for Lfx are 5.35 (strongest acidic) and 6.72 (strongest basic) and a saliva pH range was 4.5–8 \cite{25}. In patients with high salivary Lfx levels, it could be hypothesized that higher salivary concentrations could be the function of acidic salivary pH and basic drug pKa values that permitted swift transfer of Lfx from plasma to saliva and ionization thereafter. However, due to the unavailability of actual drug pKa data and unbound Lfx fraction in plasma for individual patients, we couldn’t attribute salivary pH alone to explain the variabilities in salivary Lfx concentrations. In addition, patient hydration state is thought to influence parotid salivary flow rates and in turn saliva derived drug concentrations. As saliva mainly constitutes water (97–99.5 %) originating from plasma by acinar cells, it is hypothesized that decrease in water volume due to dehydration would result in loss of salivary production \cite{28}. Fischer and Ship reported that dehydration significantly decreases the salivary output \cite{29} but could not establish a strong correlation between biological markers of hydration (haematocrit, haemoglobin, serum sodium, plasma protein, creatinine, serum and urine osmolality) and salivary output, in their study \cite{30}. Therefore, influence of hydration/dehydration status on salivary Lfx concentrations needs to be studied. Furthermore, presence of active transport channels might have contributed to high salivary concentrations, which have been studied for some peptides like insulin but not for Lfx yet and needs to be validated\cite{27}.

A noteworthy finding of our study was that Lfx in saliva does not accurately predict its plasma levels, due to variable S/P ratios at different months of treatment and large inter-individual variability in Lfx saliva concentrations CV\% (min, max) of 44.90 % and 94.29 %. Furthermore, during the second month of treatment, high inter-individual
variabilities were observed at mean t4 concentrations in both matrixes (Figure 1), cause of which could not be identified since the clinical study procedures were uniform and patients were on the same regimen for at least first two months of treatment.

This observation is not surprising as anti-TB drugs (levofloxacin, moxifloxacin, isoniazid, rifampicin and linezolid among others) exhibit high rates of PK variability even in plasma. Moreover, alternative matrices for TDM such as dried blood spots and saliva rarely have the level of precision that plasma-based assessment possess. Despite the limitations, the potential utility of saliva in semi-quantitative testing remains strong. Patients with Lfx Cmin below 2 mg/L in saliva were at the risk of lower plasma AUC0–24 (Figure 4). These patients are likely to benefit from semi-quantitative saliva based TDM in resource limited settings. However, this simple and non-invasive saliva based TDM may present few a challenge. First, the sampling procedure using salivette introduces variability in recovery depending on the type of cotton rolls used (plain cotton swab, cotton swab impregnated with citric acid, and synthetic cotton swab). We found that around 30 % of Lfx was sorbed to plain cotton rolls used for collection of saliva samples. Therefore, the saliva sample collection procedure should be standardized and well-controlled. The salivette technique further requires centrifugation for recovery of collected saliva. Alternatively, saliva samples could be collected by compressing the cotton roll in a syringe equipped with membrane filter (20). Second, it will not be feasible to analyze Lfx levels in saliva by advanced LC-MS/MS in resource limited settings. It has been prior suggested that patients at risk of low FQ exposure can be distinguished from those with normal/high exposure by a semi-quantitative point of care test such as spectrophotometer/thin-layer chromatography at a local level (31). The early pre-selection using semi-quantitative testing will act as a gate-keeper, only selecting patients at risk to offer TDM with expensive high-performance liquid chromatography technique at regional level, thereby optimally allocating resources from already depleted TB programs (10, 31). Therefore, development of a simplified, affordable, point-of care tool for determination of Lfx levels in saliva should be a priority. Third, thermal instability of anti-TB drugs and the need of refrigeration and cooling conditions for transportation might be an issue. We recently investigated the impact of high temperature
exposure on stability of Lfx and found that it was stable at 50 °C for 10 days. This is advantageous, as it precludes the cooling requirements for transportation of samples to the laboratories for TDM. Preferably, in remote settings, dried blood spots sampling could be a feasible option due to longer stability at room temperature and transportation option by regular mail but still requires the advanced LC/MS-MS for analysis. Another attraction in the field of alternative sampling could be dried saliva spots but requires sensitive high cost equipment, analytical method development and validation, and long-term stability testing at higher temperatures.

In this study, we could not use the Bland Altman method for graphical representation of mean and 95% (SD) limits of agreement between Lfx concentrations in plasma and saliva. The one sample t-test showed that the log differences between saliva and plasma concentrations were significantly different (p <0.05) from 0, which violates one of the assumptions of Bland-Altman analysis.

There were some limitations in our study. First, the sample size of 23 was rather small to explain the observed high Lfx inter- and intra-patient variability in saliva compared to plasma. To explain this effect in saliva, studies with sample size that ensures statistical power of more than 80% will be needed. Second, different predictors of salivary Lfx concentrations such as salivary flow rate were not studied. Despite the limitations, salivary Lfx concentrations could contribute as a valuable semi-quantitative pre-selection tool to identify patients’ sub-groups eligible for TDM using dried-blood spot. Patients with Lfx C_{min} below 2 mg/L in saliva could benefit from TDM due to the risk of lower plasma AUC_{0–24}.

In conclusion, this study could not demonstrate any significant relationship between plasma and saliva Lfx levels. Although Lfx penetrated in saliva, the variability in individual saliva-to-plasma ratios limits the use of saliva as a valid substitute for plasma. Despite the limitations, our data suggest that the potential utility of saliva in semi-quantitative testing remains strong. Patients with Lfx C_{min} below 2 mg/L in saliva are likely to benefit from semi-quantitative saliva based TDM in resource limited settings.
REFERENCES


(7) Alffenaar JC, Gumbo T, Aarnoutse RE. Acquired drug resistance: we can do more than we think! Clin Infect Dis 2014;60(6):969–970.


Evaluation of saliva as an alternative sampling matrix for TDM of levofloxacin


