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Early View

Research letter

Therapeutic drug monitoring using saliva as matrix: an opportunity for linezolid, but challenge for moxifloxacin

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Therapeutic drug monitoring using saliva as matrix: an opportunity for linezolid, but challenge for moxifloxacin

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Take home message: Therapeutic drug monitoring using saliva as matrix is a suitable alternative for serum therapeutic drug monitoring of linezolid, but not for moxifloxacin due to a high variability in saliva-plasma ratios.

To the editor:

The World Health Organization (WHO) has listed moxifloxacin and linezolid among the preferred "Group A" drugs in the treatment of multidrug-resistant tuberculosis (MDR-TB).[1] Therapeutic drug monitoring (TDM) could potentially optimize MDR-TB therapy, since moxifloxacin and linezolid show large pharmacokinetic variability.[1–4] TDM of moxifloxacin focuses on identifying patients with low drug exposure who are at risk of treatment failure and acquired fluoroquinolone resistance.[5, 6] Alternatively, TDM of linezolid strives to reduce toxicity while ensuring an adequate drug exposure because of its narrow therapeutic index.[1, 3, 7]

TDM is typically performed using plasma or serum samples, but other biological matrices can be considered as alternatives (e.g. saliva).[8] A benefit of saliva is the easy and non-invasive nature of sampling. Especially in high TB burden areas the option for sampling at home would be advantageous. Penetration of moxifloxacin into saliva has typically been studied in healthy volunteers, but has never been evaluated for the purpose of TDM in MDR-TB patients.[9] Only one study described linezolid concentrations in saliva and found that saliva is a suitable matrix for TDM in MDR-TB patients and that salivary concentrations can be translated to serum concentrations without the need of a correction factor.[7] The aim of this prospective study was to explore the feasibility of saliva-based TDM of moxifloxacin and to determine if earlier results of linezolid in saliva of MDR-TB patients could be confirmed.

Hospitalized adult TB patients in the Tuberculosis Center Beatrixoord (Haren, The Netherlands), who had moxifloxacin or linezolid as part of their TB treatment and had routine TDM using blood samples were eligible for inclusion. All participants signed informed consent. This study was registered at Clinicaltrials.gov (NCT03080012) and approved by the ethical review committee of the University Medical Center Groningen (IRB 2016/069).

After at least 14 days of treatment, saliva samples were taken simultaneously with plasma (moxifloxacin) or serum (linezolid) according to routine TDM schedule which generally included a

sample before and 1, 2, 3, 4, and 8 h after drug administration. All samples were stored at -80^{o}C until analysis.

To collect saliva samples, patients were asked to chew on a cotton roll after rinsing their mouth with water. Subsequently, the samples were processed using one of the following methods. Salivette (Sarstedt, Nümbrecht, Germany) in combination with centrifugation was used for non-contagious patients. Membrane filtration was applied to the saliva samples of the TB patients who still had *Mycobacterium tuberculosis* bacilli in their sputum to minimize infection hazard.[10] Salivary pH values were determined by two independent observers using pH indicator strips with pH range 4.0-7.0 and 2.0-9.0 (Merck KGaA, Darmstadt, Germany), because it could influence drug penetration into saliva.[9] Recovery of both sampling methods was determined similarly to Ghimire *et al* [11], except that moxifloxacin was tested at 1 and 3 mg/L and linezolid at 2 and 20 mg/L. Recovery was comparable for low and high concentrations. Using the Salivette, recovery was 48% (coefficient of variation [CV] 6%) for moxifloxacin or 95% (CV 3%) for linezolid and via membrane filtration 48% (CV 6%) or 98% (CV 3%), respectively. After analysis the salivary concentrations were corrected for recovery accordingly.

All samples were analysed using an updated version of our previously published liquid chromatography tandem mass spectrometry (LC-MS/MS) method.[12, 13] The LC-MS/MS method of linezolid was already cross-validated for saliva.[7] Cross-validation between plasma and saliva was performed for moxifloxacin at low (1 mg/L), medium (2 mg/L), and high (4 mg/L) concentrations as well as at lower limit of quantification (LLOQ; 0.05 mg/L). All concentration levels met the pre-set criteria for accuracy and precision (bias and CV <15%; at LLOQ both <20%).

Area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) in saliva and plasma/serum was calculated using noncompartmental pharmacokinetic analysis (MWPharm version 3.82, Mediware, Groningen, The Netherlands). C_{max} was defined as highest observed concentration and T_{max} as corresponding time of C_{max}. Two different saliva-plasma/serum ratios were calculated; one used the paired drug concentrations, while the other compared AUC_{0-24} in both matrices. Passing-Bablok

regression and Bland-Altman plots (Analyze-it 4.81; Analyze-it Software Ltd., Leeds, United Kingdom) were used to analyse results.

Patient characteristics, pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-24}), and saliva-serum/plasma ratios are shown in Table 1. All patients on linezolid did also receive moxifloxacin. Individual linezolid concentration-time curves in saliva versus serum were similarly shaped and T_{max} in saliva was not delayed, which suggested that penetration of linezolid into saliva is fast. Passing-Bablok analysis showed a linear regression line of saliva concentration = 0.389 + 0.680*serum concentration with 95% confidence interval (CI) of intercept -0.14 to 1.06; 95% CI of slope 0.60 to 0.76, r=0.954, and p=0.519. Bland-Altman demonstrated a mean (95% CI) saliva-serum concentration ratio of 0.76 (0.70-0.82). In general, the linezolid saliva-serum paired concentration ratio was considerably constant at 0.6-0.8 (range 0.25-1.29). Saliva-serum AUC_{0-24} ratios were even less variable with a median of 0.81 (range 0.54-0.96). However, we found a lower saliva-serum ratio than before,[7] which could be caused by differences in sampling method, processing or storage. Because linezolid efficacy is related to the ratio of AUC_{0-24} to minimal inhibitory concentration (AUC_{0-24}/MIC), it is recommended to collect multiple saliva samples to calculate AUC_{0-24} in saliva and afterwards translate to plasma AUC_{0-24} using a correction factor of 1.2. Based on these results, salivary TDM of linezolid indeed might be feasible and is ready for testing in a high burdened TB setting. Although LC-MS/MS was used in our study, HPLC-UV could be a suitable alternative in less resourced settings. Simple point-of-care tests in saliva, centralized drug analysis, stability studies for transport at room temperature conditions, and cross-validation of existing analytical methods in saliva may improve feasibility.[8]

Moxifloxacin paired saliva-plasma concentration ratios were highly variable with a range of 0.15-2.81 (median 1.00) which does not favour saliva as a sampling matrix for TDM. Passing-Bablok showed a linear relation of saliva concentration = -0.620 + 1.49*serum concentration, 95% CI intercept -0.97 to -0.33, 95% CI slope 1.32 to 1.74, r=0.796, and p=0.103. As moxifloxacin saliva-plasma concentration ratios were not normally distributed according to Shapiro-Wilk test (p=0.0003), Bland-Altman

analysis could not be used. Unfortunately, saliva-plasma AUC_{0-24} ratios showed similar results (range 0.30-2.00), but the underlying cause remains unclear. Both inter-individual as well as intra-individual variation was observed. No effect of salivary pH on saliva-plasma ratios of moxifloxacin could be detected. A limitation of our study was that we did not measure the unbound concentrations. Therefore, variation in protein binding could have affected the saliva-plasma ratios. Interestingly, salivary concentrations higher than plasma concentrations were observed suggesting possibilities of active transport in addition to passive diffusion.[9] Moxifloxacin also shows excellent penetration into diseased lung tissue with a median free-tissue/free-serum ratio of 3.2.[14] It would be interesting to investigate whether salivary concentrations are related to tissue concentrations at the site of infection and if penetration is driven by similar mechanisms. If closely related, it might be possible to determine infection site moxifloxacin concentrations without the need of invasive tissue sampling. Clearly, it is the free drug concentrations at the site of action that is predictive of treatment efficacy, while plasma moxifloxacin concentrations serve not more than proxy markers.

Salivary TDM could be an alternative method for traditional linezolid TDM using plasma or serum, and future studies can focus on improving the feasibility. However, for moxifloxacin our data does not support saliva as suitable matrix for TDM using the described method. Future studies should investigate moxifloxacin protein binding, salivary flow, and transport mechanisms to gain more insight in the feasibility of moxifloxacin TDM in saliva. As shown before for amikacin [15], saliva will likely not be a universal but only a selective matrix for TDM of anti-TB drugs.

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Table 1. Characteristics of the linezolid and moxifloxacin study populations, pharmacokinetic (PK) parameters in serum/plasma and saliva, and saliva-serum/plasma ratios using paired concentrations as well as AUC_{0-24}.

	Linezolid (n=7)	Moxifloxacin (n=15)
Study population		
Male [n(%)]	6 (86%)	11 (73%)
Age (years)	44 (37-55)	34 (25-55)
Bodyweight (kg)	67.1 (60.5-68.4)	67.1 (57.5-70.5)
Creatinine concentration (µmol/L)	73 (72-90)	(72 (63-90)
Dose (mg/kg)	8.85 (7.42-9.93)	5.96 (5.68-7.08)
Serum/plasma PK[a]		
C_{max} (mg/L)	12.45 (8.84-15.78)	2.28 (1.62-2.80)
T_{max} (h)	3 (2-4)	2 (1-2)
AUC_{0-24} (mg*h/L)	119.4 (116.2-128.2)	21.3 (15.8-31.0)
Saliva PK		
C_{max} (mg/L)	7.93 (7.55-12.38)	3.20 (2.51-4.25)
T_{max} (h)	3 (2-3)	2 (1-2)
AUC_{0-24} (mg*h/L)	93.6 (91.7-108.0)	21.3 (13.7-28.3)
Saliva-serum/plasma ratio[a]		
Paired concentration ratio	0.76 (0.64-0.85)	1.00 (0.68-1.35)
AUC_{0-24} ratio	0.81 (0.74-0.88)	0.89 (0.61-1.14)

All parameters are presented as median (interquartile range) unless stated otherwise.

[a] Serum for linezolid and plasma for moxifloxacin.