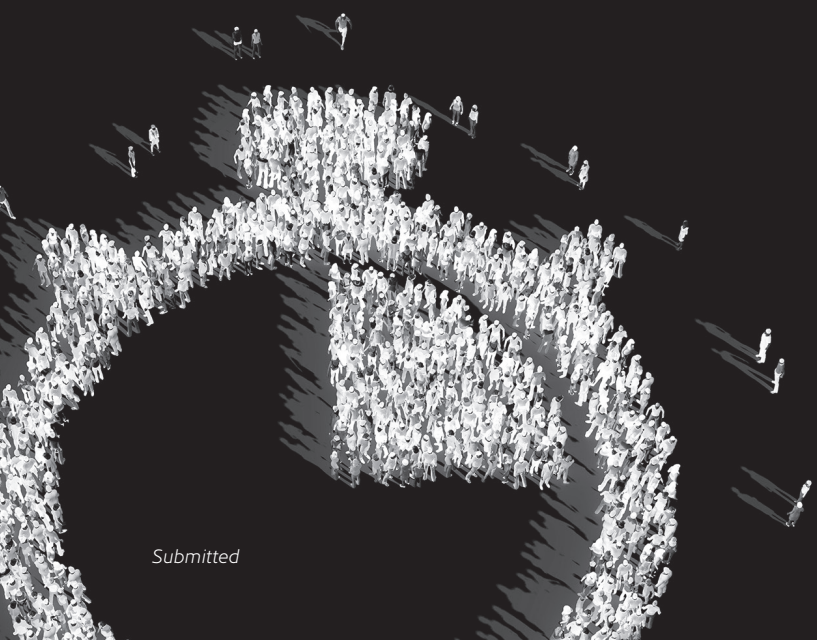
Limited Sampling Strategies for Therapeutic Drug Monitoring of Co-trimoxazole in the treatment of Multidrug-Resistant Tuberculosis



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ABSTRACT

Multidrug resistant TB, caused by *M. tuberculosis* resistant to rifampin and isoniazid, is emerging urging the need to explore alternative treatment possibilities. Sulfamethoxazole, one out of two components of co-trimoxazole, has been shown to be active against (MDR-)TB in vitro. However, the pharmacokinetics of sulfamethoxazole is rather unknown. A limited sampling model could help in studying the pharmacokinetics of sulfamethoxazole.

A prospective clinical trial was performed with twelve patients with TB. All patients were administered 960 mg co-trimoxazole (800 mg sulfamethoxazole), blood samples were frequently withdrawn and analysed. A limited sampling strategy was built using Monte Carlo simulations and with linear regression in order to estimate the area under the curve ($AUC_{0\text{-}24h}$). Monte Carlo simulations resulted in limited sampling strategies 2 and 3 hours post-dose (R2 = 0.61, prediction bias = 0.16%, RMSE: 1.5%), while linear regression resulted in a 6-hours post dose optimal sampling strategy (RMSE: 3.5%).

With these limiting sampling strategies, the $AUC_{0\text{-}24h}$ can easily be estimated using the proposed linear regression formula, or with Bayesian pharmacokinetic software. These strategies can be used in further clinical studies exploring PK/PD of co-trimoxazole in the treatment of TB.

INTRODUCTION

Multidrug resistant tuberculosis (MDR-TB) is defined as tuberculosis caused by *M. tuberculosis* (Mtb) resistant to the first-line drugs isoniazid and rifampin.[1] Unfortunately, the prevalence of MDR-TB is increasing worldwide and resistant to second line drugs, such as amikacin and kanamycin, is emerging with an estimated 46.000 newly developed cases of extreme drug resistant TB (XDR-TB) worldwide in 2014.[2] For this reason the development of new or reinvented antimicrobial drugs active against Mtb needs to be intensified. The efficacy of antimicrobial drugs, such as co-trimoxazole, already in use to treat other bacterial infections can and should also be evaluated for *M. tuberculosis*. Co-trimoxazole consists of two antimicrobials, namely sulfamethoxazole and trimethoprim in a 5 to 1 ratio. In vitro, Mtb is susceptible for sulfamethoxazole, with a mean inhibitory concentration (MIC) of 9.5 mg/L,[3-5] indicating potential use for the treatment of TB. Moreover, co-trimoxazole is inexpensive and available worldwide.

The World Health Organisation (WHO) recommends the use of 960 mg co-trimoxazole in patients with TB that are concurrently infected with HIV,[6] since randomised controlled trials proved that the mortality in patients with HIV and TB decreased while receiving co-trimoxazole.[7-9] In addition, co-trimoxazole may prevent the development of TB,[10] particularly in patients without previous antiretroviral therapy, indicating that co-trimoxazole has an effect on Mtb growth .

Sulfamethoxazole shows a concentration-dependent killing.[11] We therefore assume that the efficacy predicting parameter is the concentration of sulfamethoxazole under the curve in 24 hours ($AUC_{0\text{-}24h}$) or unbound concentration ($fAUC_{0\text{-}24h}$) divided by the MIC ($AUC_{0\text{-}24h}$/MIC).[11] The $AUC_{0\text{-}24h}$/MIC ratio for in the treatment of tuberculosis should be determined in a hollow-fiber model.[12,13]

To date the use of co-trimoxazole should be limited to those patients infected with Mtb with an extensive resistance pattern but with susceptibility to co-trimoxazole. Therapeutic drug monitoring is helpful to optimize drug exposure in patients with limited treatment options.[14] However, acquiring full plasma curves is costly and burdensome for patients involved. In addition, it is difficult to obtain full serum curves in the treatment of TB in an outpatient clinic setting.[15]

This makes it expedient to develop a limited sampling strategy using the population pharmacokinetic model. Using a limited sampling strategy, only few blood samples are needed to reliably predict the AUC. This approach is less burdensome and patients in outpatient clinics do not have to stay for a full pharmacokinetic curve.

The objective of this study is therefore to develop a limited sampling strategy of patients using 960 mg co-trimoxazole.

PATIENTS AND METHODS

Study population

Two data sets were used for this study. Data of patients who participated in an earlier prospective PK study were selected for development of a limited sampling model.[16] In brief, patients with culture-confirmed drug sensitive tuberculosis and aged between 18 and 65 years participated in that study. After 4-6 days administration of co-trimoxazole 960mg once daily blood samples were withdrawn at 8 different time points. The local ethical committee granted ethical clearance and written informed consent was obtained from the participants. Data of patients receiving co-trimoxazole as part of their MDR-TB treatment were used as external control for the developed limited sampling model. TDM was routinely performed in MDR-TB patients and the need for written informed consent was waived due to the retrospective nature of that study. More detailed data on this study population and the outcome of treatment was presented earlier.[16]

In both studies sulfamethoxazole and sulfamethoxazole-N-acetyl plasma concentrations were determined using a validated LC-MS/MS method.[17]

Limited sampling strategies

The pharmacokinetic population model described earlier was used for the development of the limited sampling strategies.[16] With the use of MW/Pharm 3.81 (MediWare, The Netherlands), Monte Carlo simulations were performed in order to calculate blood concentrations for 1,000 patients 1-8 hours after intake. Only strategies with a minimum sample interval of 1 hour, maximal 3 samples in total, a maximum total sampling period of 8 hours, a RMSE <10% and a correlation coefficient >0.7 were evaluated.

Furthermore, we evaluated possible limited sampling strategies using simple linear and stepwise multivariate linear regression. Non-linear logarithmic regression was also performed but yielded similar or less accurate results. The latter was used in order to assess limited sampling strategies involving more than one sample time. Time points were included when statistically significant ($P < 0.05$). Afterwards, the RMSE was calculated by comparing the calculated $AUC_{0\text{-}24h}$ of all 12 patients using the limited sampling strategy with the $AUC_{0\text{-}24h}$ resulting from the model calculations.

Furthermore, an external validation was performed using data from an earlier study.[18] The time points were entered in the limited sampling models, and the RMSE in predicting $AUC_{0\text{-}24h}$ was calculated.

Statistics

The predictive value of the limited sampling strategies for the $AUC_{0\text{-}24h}$ were evaluated using the RMSE (%) and by constructing a Bland-Altman plot. Predictive value of this model for the earlier population was evaluated using the RMSE. Pharmacokinetic parameters were compared

using Wilcoxon Signed Rank Tests using SPSS 20 (SPSS, Virginia, IL). Plots were constructed using SigmaPlot 12.0 (SigmaPlot, San Jose, CA).

RESULTS

Limited sampling strategies

Twelve patients participated in the prospective pharmacokinetic study. The median age was 31 years (IQR 26 - 52) with a median weight of 61.5 kg (IQR; 56.3 – 67.2) and a median height of 175 cm (IQR; 168.2 – 180.0). The measured serum creatinine varied from 48 – 83 µmol/L. The Monte Carlo simulations indicate various sampling times which met the conditions of an RMSE <10% and a correlation coefficient >0.7 (results shown in *table 1*). Considering only one sampling point, sampling 2 hours after intake provides the best estimation of the $AUC_{0\text{-}24h}$ with a R of 0.78 (R2 = 0.61) with a prediction bias of 0.16% and a RMSE of 1.53%. When sampling 2h and 3h post-dose, the estimation is slightly improved with a R of 0.90 (R^2 = 0.81) and a prediction bias and RMSE of 0.11% and 1.05%, respectively.

Table 1. Limited sampling strategies calculated by Monte Carlo simulations

Time point(s) of sampling post-dose	R	Prediction bias (%)	RMSE (%)
2 h	0.78	0.16	1.53
3 h	0.76	0.43	1.61
4 h	0.71	0.69	1.84
2 and 3 h	0.90	0.11	1.05
2 and 4 h	0.87	0.17	1.20
2 and 5 h	0.87	0.18	1.21
2 and 8 h	0.85	0.22	1.28
2 and 6 h	0.84	0.22	1.31
2 and 7 h	0.84	0.23	1.32
1 and 2 h	0.84	1.57	1.34
1 and 3 h	0.84	0.28	1.35
3 and 4h	0.84	0.34	1.36
3 and 5 h	0.84	0.34	1.37

The $AUC_{0\text{-}24h}$ prediction based on only one plasma sample was evaluated by simple linear regression. The lowest CV(RMSE) is expected when sampling at 6 hours post administration (3.53%). Sampling at 4, 5 and 8 hours post-dose results in a CV(RMSE) of 13.0%, 11.5% and 8.88%, respectively (as shown in *table 2*). Stepwise multivariate linear regression with all sampling times between 1 and 8 hours post-dose resulted in only one linear model in which both time points were significant (P < 0.05). This strategy, including both T = 4 and T = 6 samples, slightly improved the single sample estimation at T = 4 or T = 6 separately, with a CV(RMSE) of 2.3% with an adjusted R^2 of 0.992 (*table 1*).

Table 2. Limited sampling strategies calculated by simple linear regression (one sampling time) or stepwise multivariate linear regression (>1 sample times).

Time point(s) of sampling post-dose	R	RMSE (%)	Regression formula
4 h	0.882	13.0	AUC = 12.383 + 11.948 * T4
5 h	0.909	11.5	AUC = 40.822 + 11.775 * T5
6 h	0.992	3.53	AUC = 2.615 + 14.731 * T6
8 h	0.947	8.88	AUC = 63.267 + 14.573 * T8
4 and 6 h	0.997	2.27	AUC = -18.571 + 12.535 * T6 + 2.2402 * T4

Retrospective validation

The strategy with sampling 4 and 6 hours post-dose by linear regression was evaluated on the data from the earlier published retrospective study.[18]

The median deviation from the observed AUC0-24h was -14.2% (range: -19.7 – 7.9%). A Bland Altman plot of the observed AUC vs. the AUC calculated based on T=4 and T=6 from both the prospective and retrospective study is shown in *figure 1*. All but one point are within the limits of agreement. The clinical relevance of this one outlier is low, since the deviation of the AUC was approx. 20% and will most probably not result in any change in clinical intervention to change the dose.

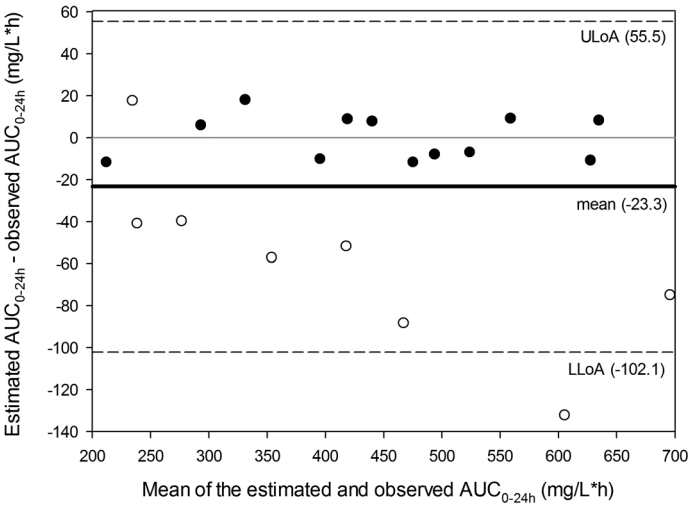

Figure 1. Bland Altman plot of the observed AUC and the AUC calculated with the proposed linear regression formula based on the sulfamethoxazole concentration T=4 and T=6 hours post-dose. Filled dots: patients from the prospective study, open dots: patients from the retrospective study. ULoA: upper limit of agreement, LLoA: lower limit of agreement.

DISCUSSION

This is the first paper describing limited sampling strategies for the estimation of the sulfamethoxazole $AUC_{0\text{-}24h}$. We presented the strategies resulting from Monte Carlo simulations and from simple and multiple linear regression. Both techniques indicate that the $AUC_{0\text{-}24h}$ can be estimated with the use of one, or preferably two serum samples.

Limited sampling models based on Bayesian estimations and based on multiple linear regression for anti-TB drugs have been used before in order to estimate the $AUC_{0\text{-}24h}$.[19,20] It has been shown that sampling at 1, 4 and 6 hours post-dose resulted in a reliable estimation of the $AUC_{0\text{-}24h}$ for rifampin, isoniazid, pyrazinamide, ethambutol and moxifloxacin simultaneously based on multiple linear regression. The limited sampling strategy for sulfamethoxazole, as proposed in this paper, fits in this strategy with sampling points at 4 and 6 hours post-dose. In addition, the limited sampling models for both aminoglycosides amikacin and kanamycin based on Bayesian estimation resulted in strategies including 1, 4 and 6 hours post-dose. Therefore, sulfamethoxazole $AUC_{0\text{-}24h}$ can also be estimated using the blood samples withdrawn for aminoglycoside therapeutic drug monitoring.[19]

Unfortunately, the pharmacokinetic/pharmacodynamic (PK/PD) target of sulfamethoxazole

in the treatment of TB is still unclear. As pointed out before, it seems reasonable to assume that the $AUC_{0\text{-}24h}$ is an efficacy and toxicity predicting parameter.[11] The proposed limited sampling model could be a tool helpful in clinical trials to estimate drug exposure to be able to correlate drug with outcome.

The Monte Carlo simulations indicate that sampling 2 and 3 hours post-dose provides the best estimation of the $AUC_{0\text{-}24h}$. By using Monte Carlo simulations, the variability is expanded which improves the selection of the most optimal sampling strategy. In addition, the predicted RMSE is lower using these proposed limited sampling strategies than using the linear regression formulas. However, in order to estimate the $AUC_{0\text{-}24h}$ of individual patients, specialized pharmacokinetic software equipped with a population pharmacokinetic model and Bayesian forecasting is needed. With this software, estimation of the $AUC_{0\text{-}24h}$ based on two serum samples is reliable and robust and can therefore be used in clinical studies and patient care.

The concentration of sulfamethoxazole on T=4 and T=6 post-dose are highly correlated with the $AUC_{0\text{-}24h}$. The $AUC_{0\text{-}24h}$ calculated based on samples withdrawn T=4 and T=6 hours after administration can therefore easily be predicted with the use of the proposed linear regression formula with low RMSE. This strong correlation suggests that this formula could be used further prospective studies and clinical care.

We also validated this linear regression method on a set of patients included in a retrospective study.[18] All calculated AUC values were within 20% of the observed AUCs from this retrospective cohort, indicating the clinical applicability of this method. However, almost all $AUC_{0\text{-}24h}$ are underestimated with the proposed limited sampling strategy. It should be noted that the majority of the patients in the retrospective cohort was on 480 mg co-trimoxazole instead of 960 mg. In addition, the retrospective study consisted of patients with MDR-TB. The difference in pharmacokinetics between the retrospective cohort and the current cohort may be caused by the difference in study populations – e.g., participants with different disease severity or underlying conditions, or drug-drug interactions. The co-administration of rifampicin, a potent CYP3A4 inhibitor, in the prospective study could explain the underestimation of the $AUC_{0\text{-}24h}$ in the cohort which received a lower dosage co-trimoxazole.

Ideally, the limited sampling strategies are combined with the sampling strategies developed for first-line and second-line anti-TB drugs.[19-21] In this manner, therapeutic drug monitoring of the anti-TB drugs could be combined with only limited serum samples needed. When the serum samples can be collected, transported and analysed using dried blood spots, therapeutic drug monitoring could also be available in developing countries.[22-24]

In conclusion, with the proposed Bayesian limited sampling strategies are suitable for clinical studies exploring PK/PD of co-trimoxazole and for therapeutic drug monitoring of sulfamethoxazole in MDR-TB patients.

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