Exploring strategies to individualize treatment with aminoglycosides and co-trimoxazole for MDR Tuberculosis
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Tuberculosis (TB), an ancient infectious disease, is an emerging threat with 1.8 million deaths in 2015 globally. TB is caused by *Mycobacterium tuberculosis*, a pathogenic bacterium from the Mycobacteriaceae family. Effective TB treatment requires a specific combination of first line, oral antibiotics: isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E). This regime means two months of these four antibiotics (2HRZE), followed by four months of only isoniazid and rifampicin (4HR) totalling to 6 months of treatment (2HRZE+4HR).

However, resistance to isoniazid and rifampicin, defined as multidrug resistant TB (MDR-TB), is an ongoing problem in the fight against *M. tuberculosis*. MDR-TB requires intensive treatment with at least four to six antibiotics: all remaining first line antibiotics, an oral quinolone and an injectable (the aminoglycosides amikacin or kanamycin or the glycopeptide capreomycin). Unfortunately, both amikacin and kanamycin come with severe side effects e.g. nephrotoxicity and irreversible hearing loss affecting the vocal frequency area. These side effects occur in 20 to 40% of all cases and causes lower treatment adherence, resulting in resistance against these aminoglycosides. Regarding hearing loss, it is proposed that the occurrence is related to the concentration profile of aminoglycosides in serum, meaning the area under the curve seems to correlate with the occurrence of side effects.

The emergence of resistance to first-line and second-line antibiotics urges the need for new antibiotics against TB. However, development of new antibiotics is expensive and requires years of development. Co-trimoxazole, a relatively old antibiotic, is currently topic of renewed interest since sulfamethoxazole, one of the two components of co-trimoxazole, showed in vitro activity against *M. tuberculosis*. Therefore, we performed a prospective study in order to establish sulfamethoxazole population pharmacokinetics in patients with *M. tuberculosis* for further dose finding studies.

In chapter 2, we reviewed the available evidence on the efficacy and toxicity of amikacin and kanamycin in the treatment of tuberculosis. We found that performing hearing tests increases the reported hearing loss prevalence and concluded that in many studies hearing loss prevalence might be underestimated. In addition, we found that the pharmacokinetics of amikacin and kanamycin are highly variable, indicating the importance of performing TDM.

In chapter 3, we studied the in vitro susceptibility differences between amikacin and kanamycin. Although amikacin and kanamycin are administered in the same dose and show the same pharmacokinetics they have different EUCAST breakpoints suggesting that the two drugs have a different minimal inhibitory concentration (MIC). The aim of this paper was to investigate any possible differences in MIC between both aminoglycosides. Using the direct concentration method, a concentration range of amikacin, kanamycin and capreomycin (0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 16.00, 32.00 and 64.00 mg/L) was tested against 57 clinical *Mycobacterium tuberculosis* strains. The results indicate that amikacin is more active against *M. tuberculosis* than kanamycin and capreomycin using the absolute concentration method. The impact of this difference on clinical outcome in daily practice requires a prospective study including pharmacokinetic and pharmacodynamics evaluations.

In chapter 4, we designed a bioanalytical method in order to quantify both amikacin and kanamycin in serum. A new method of analysis is desired since the commercially available immunoassay is unable to quantify kanamycin and the detection limit for amikacin is relatively high, making reliable quantification of trough levels impossible. A new LC-MS/MS method was developed, using apramycin as internal standard. This new method was validated in accordance with the FDA guidelines. The lower limit of quantification (LLOQ) of the LC-MS/MS method was low in comparison to the immunoassay (0.25 mg/L vs. 1.5 mg/L). No statistical significant difference was found between the analytical results of the LC-MS/MS and the immunoassay (Architect) method. This LC-MS/MS method is suitable for analysing both amikacin and kanamycin.
In chapter 5, we developed an bioanalytical immunoassay to analyse kanamycin in blood. This method was tested in a concentration range of 0.3 – 80.0 mg/L for inaccuracy and imprecision. In addition, the analytical results of the immunoassay method were compared to an LC-MS/MS analytical method using Passing and Bablok regression. No significant cross-reactivity with other antimicrobials and antiviral agents was observed. The results of the modified immunoassay method were comparable with the LC-MS/MS analytical outcome. This new immunoassay method enables laboratories to perform therapeutic drug monitoring of kanamycin without the need of complex and expensive LC-MS/MS equipment.

In chapter 6, we developed a population pharmacokinetic model of amikacin and kanamycin combined. The pharmacokinetic model was developed and validated using n-1 cross-validation. An limited sampling model was developed based on two samples obtained at 1 and 4 hours after administration with an R² of >0.99 and a bias and Root Mean Squared Error of -0.04% and 2.5%, respectively. This model in combination with the limited sampling strategy developed can be used in daily routine to guide dosing but also to assess AUC₀-2₄h in phase III studies.

In chapter 7, we evaluated the use of Therapeutic Drug Monitoring (TDM) in TB patients treated with amikacin or kanamycin in the period 2000 - 2012. Eighty patients met the inclusion criteria. The extent of hearing loss was limited and correlated with the cumulative drug dose per kg body weight during daily administration. At follow-up, 35 (67.3%) of all patients had successful outcome; there were no relapses. A randomized controlled trial should provide final proof of the safety and efficacy of TDM-guided use of aminoglycosides in MDR-TB treatment.

In chapter 8, we developed a novel liquid chromatography tandem mass spectroscopy (LC/MS-MS) method to analyse the components of co-trimoxazole, trimethoprim and sulfamethoxazole and its metabolite sulfamethoxazole-N-acetyl. This new method is expeditious due to its limited sample pre-processing and a relatively short run-time of only 3 minutes. The FDA requirements on linearity, selectivity, precision, accuracy, matrix effects, recovery and stability were met. This method is suitable for routine analysis and future prospective studies in order to establish co-trimoxazole pharmacokinetics.

In chapter 9, we described the sulfamethoxazole pharmacokinetics in patients with TB. Sulfamethoxazole possesses in vitro activity against M. tuberculosis, yet no pharmacokinetic data is available to perform a dose finding study. The objective of this study was to evaluate the PK parameters and in vitro PD data on the effective part of co-trimoxazole: sulfamethoxazole. In a prospective PK study in patients infected with drug-susceptible Mycobacterium tuberculosis (drug-susceptible TB patients) (age,>18), sulfamethoxazole-trimethoprim (SXT) was administered orally at a dose of 960 mg once daily. The area under the concentration-time curve for the free, unbound fraction of a drug (fAUC)/MIC ratio and the period in which the free concentration exceeded the MIC (fT>MIC) were calculated. The median value of the MICs was 9.5 mg/liter interquartile range [IQR], 4.75 to 9.5), and that of the fAUC/MIC ratio was 14.3 (IQR, 13.0 to 17.5). The percentage of fT>MIC ranged between 43 and 100% of the dosing interval. The PK and PD data from this study are useful to explore a future dosing regimen of co-trimoxazole for MDR-TB treatment.

In chapter 10, we propose a dried blood spot method to determine the individual sulfamethoxazole and sulfamethoxazole-N-acetyl concentration. Dried blood spots (DBS) have shown to be a reliable alternative to venous blood sampling, notably due to simple collection strategy and superior sample stability. We developed a liquid chromatography tandem mass spectrometry analysis of sulfamethoxazole and its toxic metabolite, sulfamethoxazole-N-acetyl to quantify both compounds in DBS cards. The method was validated according to FDA and EMA guidelines and clinically validated. The median difference in the area under the curve calculated
on DBS compared to plasma samples was -5.8% (IQR: -6.25 - -0.13%). This newly developed method showed to be reliable and robust and was fully validated. The stability of both compounds was sufficient to transport DBS cards from developing countries to a sophisticated laboratory to perform TDM. This method can be used in daily patient care and in future prospective pharmacokinetic studies exploring the use of sulfamethoxazole for TB treatment.

In chapter 11, we describe several limited sampling strategies for sulfamethoxazole. A limited sampling model could help to estimate the pharmacokinetics of sulfamethoxazole with only a limited amount of blood samples, reducing the patients’ burden. A limited sampling strategy was built using Monte Carlo simulations and with linear regression in order to estimate the area under the curve (AUCO-24h) based on the prospective trial as described in chapter 9. 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 AUCO-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.

In the final chapter, chapter 12, we discuss the clinical significance of this thesis and propose further steps in individualized dosing of aminoglycosides in the treatment of TB. Based on the individual pharmacokinetics and MIC of the infecting M. tuberculosis strain, an individual dose should be established to reduce the chance of toxicity, while maintaining optimal efficacy. In addition, we discussed the current knowledge on sulfamethoxazole in the treatment of TB. Further research should focus on the discovery of pharmacokinetic/pharmacodynamic targets, which can be combined with the data from our prospective study to find the optimal dose. In addition, a prospective clinical trial on the effectiveness of sulfamethoxazole in TB treatment is warranted before implementation of sulfamethoxazole in MDR-TB or XDR-TB treatment.