General Discussion
and Future Perspectives
The main focus of this thesis is the optimization of MDR-TB treatment by PK/PD analysis and dosing based on blood concentrations. We have shown that reducing the aminoglycoside dosage based on PK/PD analysis lowers toxicity while efficacy is maintained. In addition, we have raised questions on the equivalent dosage of both amikacin and kanamycin, while it appears that most \textit{M. tuberculosis} strains we tested were one log-step more susceptible to amikacin than kanamycin. Moreover, a large inter-individual MIC variation among our isolate collection was observed, which should translate into dose adjustments of aminoglycosides used.

Treatment of MDR-TB is lengthy and treatment causes many side effects. All of this results in low treatment adherence,\footnote{1} that in turn could lead to treatment failure and development of resistance to second-line anti-TB drugs. Therefore, optimizing MDR-TB treatment remains a key priority in the strategy to eradicate (MDR-)TB.

\textit{Pharmacokinetic/pharmacodynamic modelling}

The efficacy of aminoglycosides can be quantified as the $C_{\text{max}}$/MIC.\footnote{2} The large variation in MIC of amikacin and kanamycin in a collection of \textit{M. tuberculosis} strains tested should therefore have clinical implications in terms of the dosage aminoglycoside used.\footnote{3} Clearly, an increase in MIC needs to be translated in an increase in $C_{\text{max}}$ (and consequently an increase in dose) in order to reach the same $C_{\text{max}}$/MIC ratio. Individualization of therapy based on the MIC is, although this makes sense,\footnote{4} not included in the current guidelines\footnote{5,6} and is generally not performed, and the reproducibility and accuracy of MIC testing is debatable. However, the use of breakpoints in order to establish the sensitivity to aminoglycosides could therefore lead to overdosing of aminoglycosides, which comes with avoidable toxicity. We believe that binary susceptibility testing is insufficient and MICs needs to be determined quantitatively in order to individualize treatment. Unfortunately, reliable and reproducible phenotypic MIC testing is time-consuming, requires expertise, and is costly.\footnote{7} A genotypic screening capable of detecting low-level resistance (using molecular Line Probe Assay techniques or even whole genome sequencing) could be extremely helpful in order to individualize treatment based on the quantitative, rather than binary, aminoglycoside susceptibility.

The use of new platforms, such as the Sensititre plate\superscript{8} systems (Thermo Fisher Scientific, MA, US) are capable of determining the MIC in an automatic manner.\superscript{8,9} The implementation of such semi-automatic or automatic systems to facilitate MIC determination would be a valuable asset in PK/PD guided dosing of aminoglycosides. TB programmes should consider adding quantitative MIC determination appliances to their laboratories in order to determine low-level resistance, prevent further resistance development and to guide anti-TB drug dosages.

As important as pharmacodynamics, the pharmacokinetics of aminoglycosides plays also a major role in efficacy and toxicity.\superscript{10} We have shown that the pharmacokinetics of both amikacin and kanamycin can be predicted based on a limited number of blood samples. In addition, we extended the use of an immunoassay analyser to analyse both amikacin and kanamycin. This increases the availability to the analysis in third-world countries. However, immunoassay analysers are not worldwide available and transport of blood samples requires a robust cold-chain. Dried blood spots (DBS), an alternative way of sampling blood on cardboard shows superior sample stability at high temperatures and/or high air humidity.\superscript{11–13} DBS analysis has already been developed and validated for many other anti-TB drugs.\superscript{14–16} This method of analysis should be developed and validated for aminoglycosides to support pharmacokinetic analysis for remote, poor-resource settings in low- and middle-income countries.
As described in chapter 2E, aminoglycoside dosing based on serum concentration resulted in a relatively low incidence of hearing loss compared to non-serum concentration based dosing, while the efficacy was high. In this retrospective study, the dose was adjusted to reach a $\text{C}_{\text{max}}/\text{MIC}$ target of 10-20. This target is, unfortunately, not validated until today. From a study in a hollow fiber model, evidence has emerged that the $\text{C}_{\text{max}}/\text{MIC}$ ratio should be 10.13 with amikacin alone. However, possible synergy with other anti-TB drugs may lower this threshold. In this cohort only 11 (39%) of all patients achieved sputum culture conversion, which could indicate that the treatment provided was not sufficient or that treatment adherence was low. It may therefore be possible that the synergy between amikacin and the other anti-TB drugs provided was short from optimal, requiring higher $\text{C}_{\text{max}}/\text{MIC}$ ratios than needed in a well-designed and TDM-guided treatment regimen.

Dose finding studies, as performed for new drugs in general and in particular for antimicrobials, are lacking for relatively old drugs. Without the data of aminoglycoside dose finding studies, dosing is based only on an empirical basis. The use of dose finding studies in finding the most predictive PK/PD parameter by integrating antimicrobial efficacy (MIC), pharmacokinetic data (PK) and knowledge of the penetration of aminoglycosides at the site of infection is essential in the quest of reducing toxicity and maintaining efficacy. As anti-TB regimens consist of 4-6 antimicrobials, synergistic effects between different anti-TB drugs in the regimen should also be considered. Dosages of aminoglycosides and other drugs can possibly be reduced when the regimen includes also other effective anti-TB drugs such as moxifloxacin and linezolid. The $\text{C}_{\text{max}}/\text{MIC}$ target of aminoglycosides should be evaluated in combination with these agents in order to establish a more realistic $\text{C}_{\text{max}}/\text{MIC}$ target.

Prevention of hearing loss

In addition to reducing the dosage of aminoglycosides, there are more strategies to prevent aminoglycoside-induced hearing loss. The current hypothesis on aminoglycoside ototoxicity is that oxygen radicals formed in the inner ear from degradation of aminoglycosides damage sensory inner ear hair cells resulting in hearing loss. Administration of N-acetylcysteine, an anti-oxidant reduces aminoglycoside-induced hearing loss in the treatment of infections other than MDR-TB. A randomized controlled trial studying the addition of N-acetylcysteine to aminoglycoside MDR-TB treatment should be performed to observe the possible beneficial effects.

In some studies amikacin has shown to be more ototoxic than kanamycin in equivalent doses. We did not find a statistically significant difference between amikacin and kanamycin in the occurrence of ototoxicity. This might be caused by the lower dosage used in our study. It should, however, be noted that there is a difference in MIC distribution which favours the use of amikacin in terms of efficacy. It is therefore possible that the dose of kanamycin should be higher than amikacin to establish identical $\text{C}_{\text{max}}/\text{MICs}$, which could diminish the increased risk of ototoxicity due to amikacin since ototoxicity is related to the cumulative AUC. There are unfortunately no prospective trials available that compared the ototoxicity of amikacin and kanamycin on a TDM-guided dosing regimen.

It should be regular care to monitor the hearing capacity of patients treated with aminoglycosides by regular audiometry. Early hearing loss manifests at high frequencies and is therefore not easily noticeable for the patient. We have shown that studies with regular monitoring
reported more hearing loss than studies, which used patient-reported hearing loss as one of their outcomes. When early hearing loss is observed by regular monitoring, aminoglycoside-dosing schedules can be adjusted to five-weekly or thrice-weekly dosing in accordance with the WHO treatment guidelines. This could prevent or reduce a further reduction in hearing before the frequencies in the speaking-area are affected. A practical approach would be to monitor hearing loss with a smartphone and semi-professional headphones with passive noise-cancelling.

**Aerosolized aminoglycosides**

Aminoglycosides should be given intravenously or intramuscular which is inconvenient for patients. Pulmonary administration, with the use of a nebulizer, has shown to be effective in a small study.\(^2\) It is reasonable to assume that inhaled aminoglycosides reduce the transmission of tuberculosis. There are also advances made on the development of a dry powder inhaler for amikacin and kanamycin to ease the administration for the patient.\(^2\) However, large clinical trials on inhaled aminoglycosides are still lacking and are needed to establish clinical efficacy. Pulmonary administration could be administered on top of the current standardized regimens, possibly to decrease the time to sputum conversion and possibly even to shorten the intensive phase of treatment.

**Resistance development**

Unfortunately, resistance to aminoglycosides is also emerging. Treating *M. tb* without the proven effect of aminoglycosides is difficult and requires 5-6 antibiotics with mostly limited or unknown efficacy.\(^5\) Resistance to aminoglycosides is mostly caused by the production of acetyltransferase enzymes by *M. tb*, which inactivate the aminoglycosides by degrading its chemical structure. Analogous to some beta-lactam antibiotics, inhibitors of these acetyltransferase enzymes are in development.\(^25,26\) It has been proven that resistance to kanamycin could be overcome by co-administering these inhibitors *in vitro*. These acetyltransferase inhibitors would be extremely helpful in overcoming amikacin and kanamycin resistance in the treatment of extremely resistant TB.

**Co-trimoxazole – an old drug for a new indication?**

In the quest of new antibiotics, rediscovering old antibiotics for new indications is a relatively fast and cheap way to fight drug resistant microbes. In addition, the toxicity profile of old drugs is well known and much experience with this specific drug has built over the years. However, to re-use old drugs for a new indication, additional research needs to be performed. *In vitro* models, analytical methods to analyse blood samples, pharmacokinetic and pharmacodynamics studies, dose finding and clinical trials assessing safety and efficacy are required to extend a drugs’ indication list. The second part of this thesis describes extending the indication of co-trimoxazole to MDR-TB.

Sulfamethoxazole shows *in vitro* activity to drug-sensitive and MDR *M. tuberculosis*\(^27-29\). We developed an analytical method to analyse sulfamethoxazole, its metabolite sulfamethoxazole-N-acetyl and trimethoprim in serum and plasma using liquid chromatography combined with tandem mass spectrometry. We also developed a method to analyse sulfamethoxazole and its metabolite in dried blood spots and we designed multiple limited sampling strategies to calculate the sulfamethoxazole AUC with only two samples.
In addition, we performed a prospective clinical trial in order to study the pharmacokinetics of sulfamethoxazole. With this model, future predictions can be performed to find an adequate dose in the treatment of TB. However, a hollow fiber model and in vivo models are needed to establish the AUC/MIC breakpoint required to determine the most optimal dosage sulfamethoxazole. This should be performed in combination with other anti-TB drugs used in MDR-TB treatment, as synergy could be present which will lower the actual needed AUC/MIC for optimal efficacy.

**Sulfamethoxazole metabolism and toxicity**

Sulfamethoxazole is extensively metabolized by N-acetyltransferase to N-acetylsulfamethoxazole ([figure 1](#)). This metabolite is prone for crystallisation in the kidneys, causing a deteriorating renal function. In further clinical trials, the formation of N-acetylsulfamethoxazole should be monitored in order to predict and prevent toxicity.

![Metabolism of sulfamethoxazole](image)

**Figure 1.** Metabolism of sulfamethoxazole.

In addition, sulfamethoxazole is metabolized to hydroxylamine and nitroso compounds, as shown in figure 1. These compounds are haptens and are probably responsible for hypersensitivity reactions to sulfamethoxazole administrations, resulting in fever, lymphadenopathy, skin rash, hepatitis and nephritis. Frequently occurring skin reactions due to sulfamethoxazole administration are linked to proliferating T-cells due to covalently bound
sulfamethoxazole-nitroso. The nitroso compound is detoxified back into sulfamethoxazole-hydroxylamine by antioxidants, such as ascorbic acid, glutathione and cysteine. It should be noted that the metabolic route of sulfamethoxazole to sulfamethoxazole-hydroxylamine is promoted by the most frequently used anti-HIV drug ritonavir and by rifampin due to induction of the metabolic enzyme CYP2C9. Conceivably, this would imply that patients using rifampicin are more prone to sulfamethoxazole toxicity. Adding ascorbic acid or N-acetylcysteine to the sulfamethoxazole treatment could be a viable option in order to prevent hypersensitivity reactions.

Full oral drug regimen

One major problem of MDR-TB treatment is the necessity of daily intramuscular or intravenous injections with amikacin or kanamycin compromising patient adherence and complicating logistics. With the (re)discovery of linezolid, moxifloxacin and bedaquiline, all highly effective oral drugs, a full oral regimen is within reach. Although much research should still be done before introducing co-trimoxazole (or sulfamethoxazole) in standardized MDR-TB treatment regimens, sulfamethoxazole could be another effective oral drug. Regimens containing solely oral drugs, possibly with the addition of aminoglycoside dried powder inhalation, could be a viable alternative to current recommended regimens. One limitation of full oral regimens is the costs, as linezolid and bedaquiline are relatively expensive drugs in comparison with aminoglycosides. Yet, the World Health Organization and other responsible parties should provide funds to resolve this issue.

Aminoglycosides remain to be key components in MDR-TB treatment. In the newly and provisionally revised treatment guidelines for MDR-TB treatment, injectable agents are considered as group B. WHO recommendations suggest selecting 5 second line drugs for the intensive phase of treatment including one drug out of Group A: fluoroquinolones; one out of group B: injectables including aminoglycosides; and at least two out of group C: ethionamide/prothionamide, cycloserine, linezolid and clofazimine. The aminoglycosides therefore constitute one of the core components of MDR-TB treatment.

Concluding remarks

In this thesis, we provided tools to analyse and reduce aminoglycoside toxicity while maintaining efficacy. We have shown that the PK of aminoglycosides can be easily predicted with a limited number of blood samples and we developed two analytical assays to analyse these blood samples. We think that aminoglycosides can be used in a relatively safe and effective way in the treatment of MDR-TB. However, still much work needs to be done in order optimize aminoglycoside dosing. The use of in vivo and hollow-fiber models will be essential in this quest of optimizing aminoglycoside therapy. Carefully designed prospective studies are needed to validate these models and to actually assess the toxicity and efficacy of aminoglycoside dosing based on PK/PD endpoints rather than dosing based solely on the patients’ body weight. Support from national and international health authorities is essential in optimizing TB programs. For such programs to fully benefit from the precision we propose, both drug susceptibility tests and analytical tools should be in place to improve MDR-TB treatment outcome without incurring too much toxicity including hearing loss.
Chapter 12

This thesis also provides a preliminary start on the study of the efficacy and safety of sulfamethoxazole in MDR-TB treatment. We created a framework to study the pharmacokinetics of sulfamethoxazole by developing an analytical assay and we provided a limited sampling model to assess the pharmacokinetics with only one or two blood samples. In addition, we performed the first phase 2 study in humans to study the pharmacokinetics of sulfamethoxazole in TB patients. However, in vivo and hollow-fiber models are essential in repurposing sulfamethoxazole for the treatment of MDR-TB. After in vivo and hollow-fiber model confirmation of the efficacy of sulfamethoxazole, a randomized controlled trial with sulfamethoxazole as add-on to the standard treatment would provide clinical evidence for its added value, thereby establishing the therapeutic efficacy of sulfamethoxazole.

After decades of standardized treatment, we gradually agree that each patient is unique and that each disease in each patient requires individualized treatment. In the case of TB, or infectious disease in general, treatment should be individualized for the patient and also for the pathogen causing the disease. Susceptibility of M. Tb for all anti-TB drugs varies strongly among strains and resistance to current and future drugs will inevitably emerge and increase over time. The only way to manage the emerging resistance and to provide safe and effective treatment is to individualize the treatment - both for the patient and for the offending pathogen.
REFERENCES


