1 General introduction

Tuberculosis (TB) has become a historical disease in affluent regions in Europe and Northern America, virtually reaching extinction. Indeed, the prevalence of TB is relatively low in Western countries and there are diagnostic and treatment facilities in place to effectively treat TB. However, in low- and middle-income countries, TB has remained an immense global and personal health threat. The Center of Disease Control (CDC, USA) estimated that 2 billion people are infected with TB without any symptoms – the so-called latently infected pool of individuals that actually drive the current TB epidemic. Generally, some 23% of the worlds' population is latently infected but in some continents like Africa the latently infected proportion of the population is much higher.[1] A relatively small minority of these individuals subsequently progress to have active TB, with most of them developing a contagious form of TB. Indeed, in the Netherlands, contacts of TB patients had a three-fold increased chance to develop active TB, if they failed to complete preventive therapy for latent TB infection.[2] The global prevalence of TB amounts to 12 million people.[3] In 2015 alone, TB was still responsible for the death of 1.8 million people worldwide[3], urging the need to optimize and strengthen national TB programs, focusing on fast and reliable diagnostics and improving treatment in the global fight against TB.

TUBERCULOSIS

Tuberculosis (TB) is caused by tubercle bacilli – specific bacteria belonging to the genus Mycobacterium. Although most mycobacteria are hardly or not at all pathogenic, mycobacteria belonging to the small group of *Mycobacterium tuberculosis* complex are virulent and may cause TB. In humans, most cases of TB are caused by *M. tuberculosis*. Airborne droplets originating from a contagious individual while coughing or sneezing spread TB.[4] Inhaled droplets loaded with *M. tuberculosis* reach the lower airways and alveolar spaces of the newly infected individual. Specific immune cells, called alveolar macrophages and dendritic cells are able to phagocytose the micro-organisms. With the help of activated CD4+ lymphocytes, the tubercle bacilli may either be killed in the lysosome, or survive in the hostile environment of the phagosome intracellular, in a low metabolic, dormant state. Signaling molecules help to contain the infectious burden in organized cellular structures called granulomas.[5] If the immune system is unable to kill a certain part of the persistently present tubercle bacilli, an equilibrium between the immune system and *M. tuberculosis* may ensue.[6] This state is called 'latent TB' as the bacterial load remains low – i.e., under the detection limit for current bacteriological assays, bacteria remain quiescent and no symptoms are present while the individual is not contagious.

However, when the immune system is weakened by ageing, or by another infection, especially human immunodeficiency virus (HIV) infection resulting in massive reduction of CD4+ lymphocytes, TB may reactivate and TB develops resulting in severe disease or death [6].

Mycobacteria are characterized by their thick cell wall consisting of peptidoglycan linked to arabinogalactan, lipoarabinomannan and mycolic acid.[7] This tough, thick cell wall differs from other Gram-positive and Gram-negative bacteria and therefore requires specific antibiotics to kill these bacteria. TB-specific antibiotics (rifampin, isoniazid, ethambutol and pyrazinamide) are the most active antibiotics against TB and form therefore the first-line treatment.[8] A treatment with all four drugs combined for 2 months, followed by 4 months of rifampin and isoniazid therapy is needed in order to be successful[9]. The first-line treatment remains generally effective even if mono-resistance to e.g. isoniazid were present; three active drugs including rifampicin in the initial phase, and two active drugs during the four-months continuation phase thereafter are generally sufficient to sterilise the lesions and to achieve cure.

ANTIMICROBIAL RESISTANCE AND MDR-TB

Unfortunately, resistance to first-line drugs is emerging.[10,11] Multidrug resistant TB (MDR-TB), caused by *M. tuberculosis* resistant to isoniazid and rifampin, can only be treated with antibiotics less effective compared to first-line antibiotics while accompanied with side effects more often. These second-line antibiotics include aminoglycosides (amikacin and kanamycin), glycopeptides (capreomycin) and quinolones (levofloxacin, moxifloxacin, ofloxacin)[8]. The treatment of MDR-TB requires treatment well beyond 6 months; if additional resistance to fluoroquinolones or second-line injectables are present, treatment could take up to 20 months.

Aminoglycosides are injectable drugs and should be administered on a daily basis, which makes treatment complex and burdening in low-resourced settings. In addition, compliance is challenging since aminoglycoside treatment comes often with toxicity such as renal failure and hearing loss.[12–14]

All compounds in the group of aminoglycosides inhibit protein synthesis by inhibiting 30S ribosome subunit.[15] Both amikacin and kanamycin are administered in a dosage of 15 mg/kg body mass are recommended by the World Health Organization (WHO).[8] It has been suggested that an increase of the cumulative dose results in an increased prevalence of hearing loss.[14] Reducing the cumulative dose might reduce the occurrence of side effects, however, this could conceivably also impair the efficacy in eradicating *M. tuberculosis*.

THERAPEUTIC DRUG MONITORING

Despite the lack of randomized controlled trials, therapeutic drug monitoring (TDM) is commonly used to reduce toxicity and increase efficacy of aminoglycosides for infectious diseases other than MDR-TB.[16] With TDM, the dose can be adjusted based on the aminoglycoside concentration in blood. The maximum blood concentration after a dose (C_{max}) is related to the minimum inhibitory concentration (MIC).[17] This MIC is the lowest concentration of the drug able to inhibit growth of bacteria in vitro; sensitivity of the bacteria for the specific aminoglycoside is expressed in mg/L. With a lower susceptibility (and thus higher MIC), a higher C_{max} is required to achieve similar efficacy. TDM could conceivably help to monitor the C_{max} and therefore the efficacy of the TB treatment.

In addition, the area under the concentration-time curve (AUC) is associated with the occurrence of hearing loss.[14] The concentration in blood before the next dose, the trough level (C_{min}), is also related to the occurrence of toxicity. Therefore, minimization of the AUC and the C_{min} is required in order to prevent toxicity. Consequently, the balance between efficacy by maximizing the C_{max}/MIC and minimizing the AUC and Cmin is delicate. However, TDM is not implemented in TB guidelines authored by the World Health Organization and unfortunately, there are no generally accepted thresholds for the C_{max}/MIC ratio to ensure efficacy.

REDISCOVERY OF OLD DRUGS

Some new drugs for the treatment of tuberculosis are in development or approved by accelerated approval by the regulatory bodies.[18] Delamanid (OPC-67683) and bedaquilline (TMC-207) were both recently approved for the treatment of MDR-TB by both the Federal Drug Administration (FDA) and the European Medicines Agency (EMA). However, the development of new drugs is expensive and clinical experience with these drugs is lacking.

Another strategy to develop more treatment possibilities in MDR-TB treatment is by repurposing old antibiotics, which have never been used for TB before. These drugs are commonly cheap and there is a lot of clinical experience with these drugs. In addition, the drug safety profile of these old antibiotics is commonly well known. However, new antibiotics are subjected to strict pre-clinical and clinical studies in order to establish the efficacy and toxicity profile of the new drug. The pharmacokinetics and pharmacodynamics of the new investigational drug is also part of the registration file, which needs to be submitted to the regulatory offices (such as the EMA or FDA) in order to obtain market approval. For old drugs, such as co-trimoxazole, such registration requirements were not in place at the time of their introduction.

Therefore, dose-finding studies are required to establish the required dose of these antibiotics in TB patients. The pharmacokinetics and pharmacodynamics in TB patients have not been explored in formal studies. Consequently, pharmacokinetic and pharmacodynamics studies are required to find the appropriate dosing scheme for these drugs in the treatment of TB.

Co-trimoxazole is a relatively old and widely used antimicrobial agent with efficacy against many Gram-positive and Gram-negative microorganisms and molds. It consists of two components, sulfamethoxazole and trimethoprim. It has recently been shown that the sulfamethoxazole component shows in vitro activity against *M. tuberculosis*.[19] It appears therefore that co-trimoxazole might be a promising agent in the treatment of MDR-TB. Therefore, we explored the possibilities to use sulfamethoxazole in MDR-TB treatment.

AIM OF THE THESIS

The overall objective of this thesis is to optimize TB treatment by the redeveloping and repurposing of aminoglycosides and co-trimoxazole. Several tools are necessary for this optimization, starting with the development of analytical methods to quantify these medicines in serum or plasma. This to individualize aminoglycoside treatment in order to reduce toxicity and maintain efficacy.

The MIC distribution of *M. tuberculosis* for both aminoglycosides and co-trimoxazole should also be known. The objective is to establish MIC- and PK-based dosing to provide an individualized dose for each patient. This could lead to an individualized dose for aminoglycosides, in order to reduce toxicity and improve efficacy. For co-trimoxazole, information of pharmacokinetics and pharmacodynamics could lead to further study of the effectiveness. This in order to determine if co-trimoxazole could be of significant value in MDR-TB treatment.

OUTLINE OF THE THESIS

In the first part of this thesis we evaluate the pharmacokinetics and pharmacodynamics of aminoglycosides in MDR-TB. In addition, we discuss two different methods of analysis of amikacin and kanamycin in blood. Furthermore, a retrospective study is included with TDM-guided dosing in MDR-TB patients.

- In chapter 2 a literature review is performed in order to establish relationships between aminoglycoside exposure and toxicity.

- We study the susceptibility of *M. tuberculosis* to amikacin and kanamycin in chapter 3.

- In chapter 4 we describe a simple validated LC-MS/MS (liquid chromatography tandem mass spectrometry) method in order to analyse amikacin and kanamycin in serum and plasma.

- Additionally, in chapter 5 a novel method is evaluated to analyse kanamycin in serum using immunoassay analysis.

- Chapter 6 describes the pharmacokinetics of amikacin and kanamycin and we explore several limited sampling strategies to assess aminoglycoside exposure.

- In chapter 7 we perform a retrospective cohort study to assess the toxicity and efficacy of aminoglycosides a TDM-guided dosing regimen.

The second part of this thesis we explore the possible role of sulfamethoxazole in MDR-TB treatment.

- In chapter 8 we propose a validated mass spectrometry to analyse trimethoprim, sulfamethoxazole and its metabolite sulfamethoxazole-N-acetyl in serum.

- Chapter 9 describes a prospective clinical trial aimed to study the pharmacokinetics of sulfamethoxazole in MDR-TB patients.

- In chapter 10 we describe a method to analyse sulfamethoxazole and sulfamethoxazole-N-acetyl in dried blood spots.

- In chapter 11 we evaluate multiple limited sampling strategies to estimate the pharmacokinetics of sulfamethoxazole with a limited number of samples.

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