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Linezolid in multidrug-resistant tuberculosis

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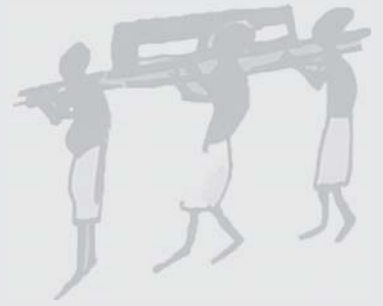
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Chapter 1

General introduction

Tuberculosis

With over 1 billion deaths in the last 200 years, tuberculosis (TB) is a bigger killer than any other infectious disease in history (1). TB is responsible for more casualties than the plague, malaria, AIDS, or cholera (1). Although TB is now considered a poverty-related illness, it may affect any susceptible individual, whether male or female, young or old, rich or poor. TB has also claimed many famous victims. Although many people know the famous victims of TB such as George Orwell, few know that Ghanaian highlife superstar Azongo “Captain Yaba” Nyaaba and Senegalese Mandinga musician Kaouding Cissoko also fell victim to TB.

More recently, over 8 million people developed TB and approximately 1.3 million people died from the consequences of TB in 2012 (2). The incidence of TB is especially high in low-income countries. However, due to permanent and temporary immigration of migrants from high prevalence countries, TB is not only a low-income country problem, but affects countries worldwide (3).

Mycobacterium tuberculosis

TB is a potentially deadly infectious disease caused by the aerobic *Mycobacterium tuberculosis*. The pathogen *M. tuberculosis* is a species of the family Mycobacteriaceae, together with other pathogens such as *Mycobacterium leprae*, *Mycobacterium ulcerans*, and *Mycobacterium avium*. In contrast to many bacteria encountered in common hospital infections, *M. tuberculosis* replicates with a minimum *in vitro* doubling time of approximately 15 hours (4). It requires a host to duplicate and has no known environmental reservoir (5).

Typically, TB is transmitted through droplets in the air. Droplets are generated by coughing or sneezing by patients suffering from pulmonary TB. With its lipid rich cell wall, *M. tuberculosis* is able to survive in air droplets, forming an infectious aerosol. When individuals inhale this aerosol containing the aerobic mycobacteria, the organisms may reach the lower respiratory tract and the pulmonary alveoli. The mycobacteria are engulfed by macrophages, such as alveolar macrophages and bronchial dendritic cells. In most cases, *M. tuberculosis* is cleared by these macrophages (6). However, mycobacteria may survive intracellularly (7, 8). When the organism survives the host immune response, a ‘balance of terror’ may ensue: the organism persists in human macrophages, in a low metabolic, slowly replicating state, referred to as ‘latent TB infection’. Before this balance is established the organism may have spread through the body via the lymphatic and the circulatory system. In small children and

in adults with a failing immune system, infection may immediately become overwhelming, with miliary TB as a result. Most individuals however develop latent infection, and never develop active disease; some 10% of individuals develop active TB disease any time following infection. The pool of latently infected individuals represents the reservoir of the organism; as much as one third of the world population is considered carrier of TB (9). *M. tuberculosis* primarily infects the lungs, and established disease predominantly presents as pulmonary TB. As explained, TB may however affect virtually every organ and tissue.

Symptoms and diagnosis

Patients with latent TB infection are infected with *M. tuberculosis* without displaying any symptoms; the bacilli have low metabolic and replicative activity, and the numbers are so low that the organisms cannot be detected by microbiological methods. Only immunological tests are able to confirm the diagnosis. When the immune system fails to control *M. tuberculosis* replication and metabolic activity, active disease may ensue with increased numbers of bacilli causing symptoms of cough, weight loss, fever, chest pain, weakness or fatigue, night sweats and coughing up of blood (10, 11).

The clinical diagnosis of TB based on a combination of symptoms, history of prior TB infection, epidemiological factors and radiographic/laboratory findings can now be confirmed by microbiological methods. These microbiological methods comprises microscopy using acid- and alcohol-fast staining procedures, culture using specific culture media, and polymerase chain reactions demonstrating the presence of specific sequences of the DNA of *M. tuberculosis*.

Multidrug-resistant tuberculosis

A worrisome fact is that an increasing proportion of TB patients happen to be infected with drug resistant *M. tuberculosis* strains. The World Health Organization (WHO) suggests that approximately 3.6% of new TB cases have multidrug-resistant strains, with much higher levels – up to 20% – in previously treated cases (12). In multidrug-resistant TB (MDR-TB) the organism is resistant to at least the classic anti-TB drugs rifampicin and isoniazid (13). For the individual patient, MDR-TB is bad news: the treatment duration required to obtain cure is increased more than three-fold, and outcome is less certain. Drug resistance is the result of selective pressure to resistant mutants in the microbial population causing the

infection; with inadequate therapy suppressing susceptible wild-type organisms, mutants may re-populate infection sites, and in the end all organisms are drug-resistant. It would help if fast molecular tests were available to timely detect and adequately target the offending organisms with a drug treatment combination that matches the susceptibility to the drug combination selected. Molecular testing using the Hain genotype MTBDRplus might give a swift preliminary result of the resistance of the isolate to rifampicin and isoniazid (14). The test is based on detecting the most common DNA mutations. As a proxy of MDR-TB, resistance to rifampicin only is now commonly used. The most widely used and studied test is probably GeneXpert-TB-RIF, a system that has been developed and validated in different settings (15, 16) and that can be used as a point-of-care test, also in settings with limited laboratory expertise available (17, 18). However, the gold standard to determine drug resistance is by analysing the minimal inhibitory concentration of a sample of mycobacteria isolated from the patient using the absolute concentration method (19).

To treat MDR-TB, the WHO recommends to design treatment regimens containing at least four drugs that are probably effective (13). Therefore physicians are forced to design treatment regimens using less effective and less well-studied 'second-line' drugs. Some are prone to elicit adverse effects, further limiting the applicability to treatment regimens.

Treatment options are divided in several WHO groups (13). Group 2 – the second line parenteral drugs or 'injectables' – is composed of aminoglycosides, e.g. amikacin and kanamycin. Group 3 are the fluoroquinolones, with very active and widely used drugs such as moxifloxacin; and the group 4 drugs including oral bacteriostatic second-line anti-tuberculosis drugs, e.g. prothionamide and cycloserine.

As a last resort, physicians are often forced to prescribe WHO Group 5 drugs such as linezolid and clarithromycin. Both drugs are described in this thesis. Linezolid and clarithromycin are drugs with unclear efficacy and are therefore not recommended for routine use in treatment regimens for MDR-TB (20). However, more knowledge on the efficacy, toxicity, tolerability, i.e. the clinical pharmacology might unleash their untapped potential.

Clinical pharmacology

New information on pharmacology in a clinical setting might contribute to the applicability of linezolid in the treatment of MDR-TB. The two main areas of pharmacology are pharmacokinetics and pharmacodynamics. Pharmacokinetics describes the movement

of the drug through the body (absorption, distribution, metabolism and elimination): it addresses the question how the organism handles the drug. Pharmacodynamics describes the action of the drug in the human host – and in case of micro-organisms – how the offending organism is targeted.

Therapeutic Drug Monitoring

These two principles, pharmacokinetics and pharmacodynamics, can be combined to perform therapeutic drug monitoring (TDM). TDM is defined by the International Association of Therapeutic Drug Monitoring and Clinical Toxicity as ‘a multi-disciplinary clinical specialty aimed at improving patient care by individually adjusting the dose of drugs for which clinical experience or clinical trials have shown it improved outcome in the general or specific populations (21)’.

TDM could perhaps improve the treatment of MDR-TB using the WHO group 5 drugs, such as linezolid. Therefore, we aimed to study the clinical pharmacology, with special focus on TDM of linezolid to optimize treatment of patients suffering from MDR-TB. More specific, we aimed to give a review of literature on pharmacokinetic drug interactions of anti-mycobacterial drugs. Furthermore, we aimed to study a potentially new pharmacokinetic drug-drug interaction between linezolid and clarithromycin and to analytically and clinically validate two new methods to analyse linezolid in oral fluid and dried blood spots obtained from MDR-TB patients. Finally, we aimed to retrospectively study linezolid exposure in relation to efficacy, safety and tolerability in the treatment of MDR-TB. Besides the aim to study pharmacokinetics of linezolid, we aimed to investigate possible pharmacodynamic interaction between linezolid and clarithromycin in MDR-TB isolates.

Outline of the thesis

Chapter 2: In this chapter, we have reviewed the literature on pharmacokinetic drug interactions of anti-mycobacterial drugs. These drug interactions are important since impact efficacy and toxicity of drugs that are part of a treatment regimen in which very few treatment options are open, especially in case of MDR-TB. Drug-drug, food-drug, and herbal medicine-drug interactions are described focusing on the effect of the interaction on the antimicrobial drug itself (antimicrobial drugs as *victim*) or on the effect of the co-prescribed drug (antimicrobial drugs as *perpetrators*).

Chapter 3: In the third chapter, we studied a pharmacokinetic drug-drug interaction between linezolid and clarithromycin based on a remarkable clinical finding from a patient admitted at the Tuberculosis Center Beatrixoord (University Medical Center Groningen, Haren, The Netherlands). As a part of routine therapeutic drug monitoring, linezolid blood concentrations were analysed. We discovered a considerable increase of the patient's linezolid concentration after co-administration of clarithromycin. In this chapter we further explored the role of drug-drug interactions of linezolid with clarithromycin.

Chapter 4: Therapeutic drug monitoring has gradually become a more widely accepted tool to optimize individual treatment regimens of anti-TB drugs. However, logistical problems with conventional drug sampling, and cold-transport of blood specimens to laboratories limit the use of TDM to research-oriented institutes. In this chapter, we aimed to develop and clinically validate a technique to overcome these problems: dried blood spot analysis of linezolid in patients with MDR-TB.

Despite “lacking the drama of blood, sincerity of sweat, and the emotional appeal of tears (22)”, another potential advantageous matrix with non invasive sampling is oral fluid. In the second part of this chapter we developed and clinically validated the analysis of linezolid and clarithromycin in oral fluid of MDR-TB patients.

Chapter 5: In this chapter, we strived to get insight in linezolid efficacy, safety, and tolerability in patients with MDR-TB, focusing on pharmacokinetics and pharmacodynamics. Published data were lacking detailed information on pharmacokinetic / pharmacodynamic targets; several recently published studies did not incorporate TDM in their study designs. In two letters to the editor, we advocated the use of TDM in clinical trials in order to generate data on an anti-TB drug that is known to show inter-patient variability in pharmacokinetics and to display drug-interactions.

This encouraged us to retrospectively analyse the data that was generated in the previous years in our TB Center. In order to enlarge our cohort, we included patients from both our Tuberculosis Center Beatrixoord (University Medical Center Groningen, Haren, The Netherlands) and from the Tuberculosis Reference Center for MDR-TB and HIV-TB E. Morelli Hospital (Sondalo, Italy). For this retrospective study, we planned to select patients that received linezolid as a part of their treatment regimen for MDR-TB and that underwent TDM. We aimed to relate linezolid efficacy, safety, and tolerability to linezolid drug exposure in MDR-TB patients.

Chapter 6: As a rule, pharmacokinetic interactions are considered disadvantageous and potentially harmful. In this chapter, we study a potentially beneficial pharmacodynamic interaction between linezolid and clarithromycin. With a lack of new anti-TB drugs emerging from the pipeline, an effort should be made to optimize treatment regimens containing existing drugs with activity against *M. tuberculosis*. One of these drugs, clarithromycin, has a controversial role in treatment regimens due to the fact that blood levels of clarithromycin measured in patients are often below minimal inhibitory concentration as determined *in vitro* in clinical isolates. One reason to incorporate clarithromycin in treatment regimens is *in vitro* synergy between clarithromycin and isoniazid, rifampicin and/or ethambutol against *M. tuberculosis* (23). In this chapter, we investigated whether linezolid and clarithromycin display *in vitro* synergy in clinical isolates of *Mycobacterium tuberculosis*.

Chapter 7 and 8: In the seventh chapter, we present a summary of the findings of previous chapters. In the General Discussion of this thesis (chapter 8), we discussed the clinical impact of the studies presented on the role of linezolid in optimizing treatment for patients with MDR-TB. We discussed the clinical pharmacology, with especial focus on TDM, of linezolid in the context of MDR-TB, and present future perspectives.

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