A quest to optimize the clinical pharmacology of tuberculosis and human immunodeficiency virus drug treatment

Daskapan, Alper

1

General introduction

Tuberculosis

Tuberculosis (TB) is an ancient infectious disease caused by the *Mycobacterium tuberculosis* (*M. tuberculosis*) bacilli. TB has scourged humankind worldwide for centuries [1]. *Mycobacterium tuberculosis* complex consists of at least nine species [2], with *M. tuberculosis* being the most virulent and most common cause of TB in humans. Like all mycobacteria (with close to 200 species), its thick cell wall is rich in lipids such as mycolic acid [3] and is therefore impermeable for basic dyes. Instead, acid-fast stains such as Ziehl-Neelsen stain, or fluorescent stains such as auramine are used and therefore *M. tuberculosis* is also described as acid-fast [4]. TB is a communicable disease and the bacillus *M. tuberculosis* is expelled in airborne droplets when contagious people cough, sneeze, talk and spit [5,6]. After inhaling *M. tuberculosis* organisms, probably many individuals fight off these organisms by their natural host barriers including the natural barrier of intact respiratory mucosal protection with ciliary transport, and without any encounter with the innate and adaptive host immune responses. If infection occurs, involving encounter with host alveolar macrophages and granulocytes that are in turn triggered by host pathogen recognition patterns recognizing pathogen-associated molecular patterns, the resulting host response may be detected by a positive result in an interferon gamma-release assay (IGRA), or a tuberculin skin test (TST). Some individuals with this positive test result may have cleared the infection, while in others infection results in progression and TB [7]; in the majority of cases, *M. tuberculosis* survives while being enveloped by macrophages resulting in latent TB infection [8]. Latent TB infection comprises a non-contagious, low metabolic state of *M. tuberculosis* in a dormant state with a low bacterial load. The risk of reactivation resulting in active TB is greatest in the first two years after exposure, i.e., after TST or IGRA turned positive [8]. The TB epidemic world-wide is maintained by reactivated TB, originating from the huge reservoir of latently infected individuals – approximately, 23% of the world population [5,8]. *M. tuberculosis* requires oxygen to grow and therefore grows most successfully in tissues with high oxygen levels, such as the lungs [1]. Although TB typically affects the lungs, in around 30% of cases TB is located in extrapulmonary sites, such as the heart, lymph nodes, intervertebral and in the central nervous system causing tubercular meningitis. Major symptoms of TB include a chronic (>weeks) cough, often productive - sometime with expectoration of blood (haemoptysis); weight loss; and fever, often reported as night sweats. Although a relatively small proportion of the people latently infected with TB develop active TB infection (5 – 15%), TB currently is the number one killer among infectious diseases, with a sad record of 1.7 million deaths is 2016 alone [5]. TB control is being burdened by poverty in highly endemic areas and overcrowding in addition to patient related contributors such as: diabetes, malnutrition, drug abuse, smoking and co-infection with human immunodeficiency virus (HIV) [9-12].

Human immunodeficiency virus

HIV co-infection is the most important predisposing factor for TB. HIV infection increases the risk of latent TB reactivation markedly [9]. The probability of developing TB is 17 – 22-fold higher for people living with HIV compared to those without HIV infection [9]. HIV/acquired immunodeficiency syndrome (AIDS) is the second deadliest infectious killer worldwide with an estimated number of nearly 1 million deaths from HIV/AIDS [5] of which approximately 400.000 co-infected with HIV/TB (figure 1). Systemic infection with HIV is commonly established by sexual transmission, transmission by intravenous drug use or by vertical (mother-to-child) transmission [13]. Since the CD4-receptor is required for HIV to penetrate and infect cells, CD4+ T helper cells are preferentially targeted by the virus [14]. Also the chemokine receptors CCR5 and CXCR4 are targeted by HIV, which are expressed on monocytes, macrophages and dendritic cells [15]. Indeed, the hallmark of HIV infection is the depletion of CD4+ T-cells thereby weakening the host immune system [16]. Immediately following infection with HIV, in the initial infection phase, there is massive decline of CD4+ T-cells; most of this CD4+ T-cell mass resides in the gut mucosa [15]. At this time point of the infection, before the antibody response has been fully initiated, viral loads typically peak [17]. After the antibody response has appeared there is a decline in HIV viral load, with ineffective viral suppression and on-going replication, usually reaching a set-point of viral load that is different in different HIV-infected individuals, but that is relatively stable over time in one particular individual [15]. This is the latent phase of the infection and if the infection is untreated it leads to further decline of the host immune system with gradual decline in CD4+ cell count in the blood stream. Eventually viral load increases with a further decline of CD4+ cells usually leading to opportunistic infections or other AIDS-defining illnesses (e.g., opportunistic tumours or AIDS-associated dementia) when the CD4+ count drops below 200 cells/μL. [18]. People latently infected with TB might develop active TB infection at any one time point during HIV infection. The two pathogens *M. tuberculosis* and HIV are known to act in synergy by potentiating each other, thereby further weakening the host immune system [16].

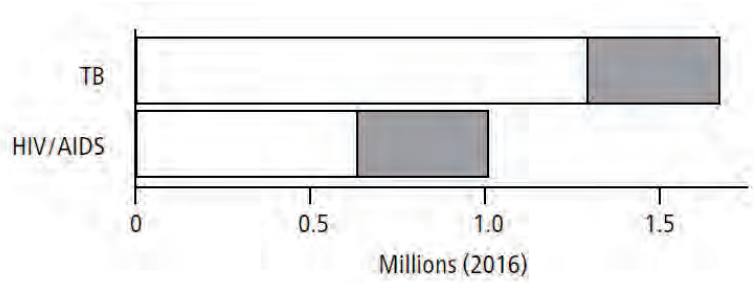

Figure 1. Estimated number of deaths from TB and HIV/AIDS in 2016; adapted from the Global Tuberculosis Report 2017; available at: *http://apps.who.int/iris/bitstream/handle/10665/259366/9789241565516-eng.pdf*. Deaths from TB among HIV-positive people are shown in grey

Historical perspective

After Robert Koch discovered and reported the cause of TB in 1882, it took several decades to find effective drugs for combating TB. In 1943, both streptomycin [19] and para-aminosalicyclic acid (PAS) [20] were discovered to be effective against TB. Although these drugs were effective, there were serious side effects and with monotherapy, drug resistant organisms emerged rapidly [21]. The next major discovery was in 1951, when isoniazid was introduced [22]. The thick cell wall and slow replication rate of *M. tuberculosis* (15 – 20h) necessitates the use of potent drugs for a long period of time [1]. In the following two decades, isoniazid, PAS and streptomycin were used for TB treatment as a triple-therapy for a period of 18 – 24 months. This combination of drugs remained the gold standard until rifampicin was introduced in 1966 [23]. Next, ethambutol was added [24] which together with rifampicin and isoniazid enabled the chemotherapy to be shortened to 9 months [24]. In the late 1970s, pyrazinamide was added to the chemotherapy and this resulted in effective treatment of 'short duration': six months of treatment resulted in excellent relapse-free cure rates [25]. Although several drugs active against TB were developed after this period, the six-month therapy containing a two-month intensive phase of rifampicin, isoniazid, ethambutol and pyrazinamide and a four-month continuation phase with rifampicin and isoniazid has remained the cornerstone of drug-susceptible TB to date [5]. The currently used chemotherapy resulted in a tremendous decline in TB incidence in the 1980s and the scientific and political interest for TB faded. No new TB drugs were developed in the next four decades. With the emergence of the HIV epidemic, there was a resurgence of TB in the 1990s [26] and with the emergence of isoniazid and rifampicin resistant strains, thereby the emergence of multi-drug resistant (MDR) TB. In response to the emergence of MDR-TB, only recently two new TB drugs (bedaquiline and delamanid) were developed in the fight against MDR-TB. In the meantime, awaiting these novel agents to be brought to the market, a strategy still currently in use was to repurpose antimicrobial agents with anti-TB activity in the treatment of patients with MDR-TB.

The history of anti-retroviral drugs to treat HIV – now referred to as combination antiretroviral therapy (cART) – only dates back to the 1980-ties. After the isolation of HIV-1 in 1983 [27], huge efforts were undertaken to control the virus. Antiretroviral drugs are designed to intervene in the HIV replicative cycle, which eventually results in an HIV ribonucleic acid (RNA) load decrease and subsequently, the recovery of the host immune system. The first drugs developed are classified within the group of nucleoside reverse transcriptase inhibitors (NRTIs) [28]. Within a short period of time the first antiretroviral drug azidothymidine was approved [28]. Monotherapy had limited efficacy, while side effects like anemia and lipodystrophia as well as drug resistance limited its use. Due to the emergence of drug resistance and limited efficacy when a single antiretroviral drug was administered, combination therapy with two NRTIs was attempted with only limited improvement in response [29]. The major break-through in HIV therapy came in 1996 with combining anti-retrovirals from different classes, such as

non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PI). For the first time, this 'highly active' combined anti-retroviral therapy resulted in a decline of drug resistance, and a steady and durable decline in viral load, with recovery of immunity with marked reduction in AIDS-related mortality. Further advancements have since been made in HIV drug therapy by the development of potent and relatively safe antiretroviral drugs belonging to the old classes, such as the PI darunavir [30].

Also, new drug classes were developed with a different mode of action; fusion inhibitors, entry inhibitors, co-receptor inhibitors (targeting HIV-1 with CCR5 tropism) and integrase strand transfer inhibitors. The drug elimination of most PIs is by hepatic elimination – and pharmacokinetics improved markedly by co-administration of a boost-drug (cobicistat or ritonavir) thereby making once daily dosing possible. Finally, fixed drug combinations reduced the pill burden and thereby, adherence to therapy markedly.

Since the advent of cART, the mortality and morbidity associated with HIV-1 infection and acquired immunodeficiency syndrome (AIDS) sharply decreased [31].

Clinical pharmacology

Clinical pharmacology is the science of drugs and their clinical use. It is based on the science of pharmacology with focus on the application of pharmacological principals and quantitative methods in clinical practice. Two key branches of clinical pharmacology are the pharmacokinetics (PK) and the pharmacodynamics (PD). Pharmacokinetics describes what the body does to the drug in terms of: absorption, distribution, metabolism and excretion. Pharmacodynamics describes what the drug does to the drug target in the body and in the specific cases of TB and HIV, it describes the targeting of the pathogens by TB drugs and anti-retroviral agents. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antibiotic that will inhibit the visible growth of a microorganism following incubation. In daily practice the MIC is used as a measure for the potency of an antimicrobial agent for a specific microorganism.

Another key factor in the efficacy of anti-infective agents is the antimicrobial PK target of the drugs. The efficacy of antimicrobial agents depends on the area under the concentration-time curve (AUC), peak concentration (C_{max}) or the time (T) in relation to the MIC (AUC/MIC; C_{max}/MIC or T>MIC ratio). For some TB drugs it has been established which antimicrobial PK target is most predictive for adequate antimicrobial killing or inhibition, but for the most first-line TB drugs there is no consensus. The PK of a drug in turn depends on several parameters: the liberation of a drug from its dosage form; drug absorption; volume of distribution; metabolism and elimination, often abbreviated to LADME. When knowledge about PK/PD

is combined, we have all data available to model effective drug treatment. We assume that such modelling provides all data necessary to understand and improve TB and HIV drug treatment. First-line TB drugs are relatively old drugs and registration studies were often not profound back then as they are nowadays; and therefore data lacking antimicrobial PK targets is often lacking. Recent studies however, combining PK and PD data of first-line TB drugs suggest that the driving factor for *M. tuberculosis* eradication is best reflected by either the AUC or C_{max} to MIC ratio [32-34].

Treatment of TB and HIV has several challenges - and success depends on many factors. There are many potential contributing factors for TB related mortality due to suboptimal drug treatment among both TB and TB/HIV co-infected patients. Factors such as non-adherence, co-morbidities (diabetes and HIV), drug-drug interactions and PK variability might lead to sub-therapeutic drug concentrations [35]. Partly due to the long duration of TB treatment and the resource-limited settings in high endemic areas, patients are faced with difficulties to comply with treatment [10]. Further, TB patients (especially those with TB/HIV co-infection) suffer from drug-drug interactions [35]. Although non-adherence is a major contributor to inadequate drug therapy, inter-individual PK variability also might lead to suboptimal therapy. Several factors cause inter-individual PK variability, including body weight [11], sex [12], pharmacogenomics [36] and comorbidities such as diabetes and HIV [12,35]. High PK variability is undesirable as high drug concentrations could lead to side effects and dose-related toxicity, while low drug exposure predisposes to prolonged treatment, treatment failure, relapse and development of drug resistance [32].

All these problems also apply for people receiving HIV drugs such as darunavir. Also here drug-drug interactions and treatment adherence play a role for treatment success. Although HIV drug treatment improved drastically, people living with HIV still encounter problems in terms of high pill burden and concomitant food intake as necessary with darunavir ingestion.

Objective of the thesis

Providing a good insight in the PK of TB drugs and antiretrovirals could contribute to improved treatment outcomes in HIV-, TB-, and TB/HIV co-infected patients. The objective of this thesis is to provide insight in the treatment of drug-susceptible TB in TB/HIV co-infected patients on the one hand and to provide tools for a tailor-made antiretroviral treatment, particularly for darunavir on the other.

Outline of the thesis

In the first part of this thesis the treatment of TB in patients with TB/HIV co-infection is evaluated.

- Chapter 2, is a systematic review which will be performed to provide insight on the effect of HIV infection on the PK of the first-line tuberculosis drugs: rifampicin, isoniazid, pyrazinamide and ethambutol. In collaboration with an international group of TB scientists, physicians, epidemiologists and pharmacists, available literature will be evaluated and relevant findings will be presented.

- In Chapter 3, we study the predictors of a prolonged TB treatment in a Dutch outpatient setting retrospectively.

- Three case-reports and letters are presented in Chapter 4a-c that discusses personalized TB drug treatment in patients with TB/HIV co-infection.

- In the second part of the thesis several tools will be explored for the optimization of HIV treatment, particularly with the antiretroviral protease inhibitor darunavir.

- In Chapter 5, we plan to prospectively evaluated the food intake and the darunavir concentrations in people living with HIV in an outpatient setting.

- In Chapter 6, we plan to develop and validate a bioanalytical method for the simultaneous determination of 14 antiretroviral drugs using liquid chromatography-tandem mass spectrometry.

- In Chapter 7 we plan to make a population pharmacokinetic model of darunavir to facilitate therapeutic drug monitoring in daily practice.

- In Chapter 8 we try to find potential risk factors predisposing for a low darunavir plasma concentration by means of a retrospective study.

- In Chapter 9, the General Discussion and the final chapter of this thesis, the major outcomes and the impact of TB and HIV research in general will be discussed from a abstract point of view and based on results from previous chapters and current developments in the field, future perspectives are proposed.

- In Chapter 10, a brief summary of the main findings of the previous chapters is given.

References

1. Lawn SD, Zumla AI. Tuberculosis. *Lancet*. 2011;378(9785):57-72.
2. van Ingen J, Rahim Z, Mulder A, et al. Characterization of mycobacterium orygis as M. tuberculosis complex subspecies. *Emerg Infect Dis*. 2012;18(4):653-655.
3. Brennan PJ. Structure, function, and biogenesis of the cell wall of mycobacterium tuberculosis. *Tuberculosis (Edinb)*. 2003;83(1-3):91-97.
4. Cudahy P, Shenoi SV. Diagnostics for pulmonary tuberculosis. *Postgrad Med J*. 2016;92(1086):187-193.
5. World Health Organization (WHO). Global tuberculosis report 2017. http://apps.who.int/iris/bitstream/10665/259366/1/9789241565516-eng.pdf?ua=1. Updated 2017. Accessed 2/8, 2018.
6. Yates TA, Khan PY, Knight GM, et al. The transmission of mycobacterium tuberculosis in high burden settings. *Lancet Infect Dis*. 2016;16(2):227-238.
7. Getahun H, Chaisson RE, Raviglione M. Latent mycobacterium tuberculosis infection. *N Engl J Med*. 2015;373(12):1179-1180.
8. Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: A re-estimation using mathematical modelling. *PLoS Med*. 2016;13(10):e1002152.
9. Getahun H, Gunneberg C, Granich R, Nunn P. HIV infection-associated tuberculosis: The epidemiology and the response. *Clin Infect Dis*. 2010;50 Suppl 3:S201-7.
10. Herrero MB, Arrossi S, Ramos S, Braga JU. Social determinants of nonadherence to tuberculosis treatment in buenos aires, argentina. *Cad Saude Publica*. 2015;31(9):1983-1994.
11. McIlleron H, Rustomjee R, Vahedi M, et al. Reduced antituberculosis drug concentrations in HIV-infected patients who are men or have low weight: Implications for international dosing guidelines. *Antimicrob Agents Chemother*. 2012;56(6):3232-3238.
12. Requena-Mendez A, Davies G, Ardrey A, et al. Pharmacokinetics of rifampin in peruvian tuberculosis patients with and without comorbid diabetes or HIV. *Antimicrob Agents Chemother*. 2012;56(5):2357-2363.
13. Shaw GM, Hunter E. HIV transmission. *Cold Spring Harb Perspect Med*. 2012;2(11):10.1101/cshperspect.a006965.
14. Swanstrom R, Coffin J. HIV-1 pathogenesis: The virus. *Cold Spring Harb Perspect Med*. 2012;2(12):a007443.
15. Lucas S, Nelson AM. HIV and the spectrum of human disease. *J Pathol*. 2015;235(2):229-241.
16. Pawlowski A, Jansson M, Skold M, Rottenberg ME, Kallenius G. Tuberculosis and HIV co-infection. *PLoS Pathog*. 2012;8(2):e1002464.
17. Rayment M, Asboe D, Sullivan AK. HIV testing and management of newly diagnosed HIV. *BMJ*. 2014;349:g4275.
18. Maartens G, Celum C, Lewin SR. HIV infection: Epidemiology, pathogenesis, treatment, and prevention. *Lancet*. 2014;384(9939):258-271.
19. FELDMAN WH, HINSHAW HC, MANN FC. Studies in streptomycin in tuberculosis; the action of streptomycin in experimental tuberculosis. *Trans Annu Meet Natl Tuberc Assoc*. 1947;42:64.

20. LEHMANN J. Para-aminosalicylic acid in the treatment of tuberculosis. *Lancet*. 1946;1(6384):15.
21. Streptomycin resistance in tuberculosis. *Br Med J*. 1948;2(4588):1025-1026.
22. SELIKOFF IJ, ROBITZEK EH. Tuberculosis chemotherapy with hydrazine derivatives of isonicotinic acid. *Dis Chest*. 1952;21(4):385-438.
23. Maggi N, Pasqualucci CR, Ballotta R, Sensi P. Rifampicin: A new orally active rifamycin. *Chemotherapy*. 1966;11(5):285-292.
24. Short-course chemotherapy in pulmonary tuberculosis. A controlled trial by the british thoracic and tuberculosis association. *Lancet*. 1976;2(7995):1102-1104.
25. Clinical trial of six-month and four-month regimens of chemotherapy in the treatment of pulmonary tuberculosis. *Am Rev Respir Dis*. 1979;119(4):579-585.
26. Murray JF, Schraufnagel DE, Hopewell PC. Treatment of tuberculosis. A historical perspective. *Ann Am Thorac Soc*. 2015;12(12):1749-1759.
27. Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science*. 1984;224(4648):500-503.
28. Furman PA, Fyfe JA, St Clair MH, et al. Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc Natl Acad Sci U S A*. 1986;83(21):8333-8337.
29. Hammer SM, Katzenstein DA, Hughes MD, et al. A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter. AIDS clinical trials group study 175 study team. *N Engl J Med*. 1996;335(15):1081-1090.
30. Scholler-Gyure M, Kakuda TN, Sekar V, et al. Pharmacokinetics of darunavir/ritonavir and TMC125 alone and coadministered in HIV-negative volunteers. *Antivir Ther*. 2007;12(5):789-796.
31. Arts EJ, Hazuda DJ. HIV-1 antiretroviral drug therapy. *Cold Spring Harb Perspect Med*. 2012;2(4):a007161.
32. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Smith P, Gumbo T. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *J Infect Dis*. 2013;208(9):1464-1473.
33. Gumbo T, Louie A, Deziel MR, et al. Concentration-dependent mycobacterium tuberculosis killing and prevention of resistance by rifampin. *Antimicrob Agents Chemother*. 2007;51(11):3781-3788.
34. Gumbo T, Louie A, Liu W, et al. Isoniazid bactericidal activity and resistance emergence: Integrating pharmacodynamics and pharmacogenomics to predict efficacy in different ethnic populations. *Antimicrob Agents Chemother*. 2007;51(7):2329-2336.
35. Alsultan A, Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis: An update. *Drugs*. 2014;74(8):839-854.
36. Chigutsa E, Visser ME, Swart EC, et al. The SLCO1B1 rs4149032 polymorphism is highly prevalent in south africans and is associated with reduced rifampin concentrations: Dosing implications. *Antimicrob Agents Chemother*. 2011;55(9):4122-4127.