Quantitative pharmacology of antimicrobials

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Section I

Introduction

Chapter 1

General introduction and scope of thesis

Introduction

Antimicrobial drugs constitute a fundamental part of modern medicine. The global rise in antimicrobial resistance (AMR), which decreases effectiveness of antimicrobial treatments, poses a major threat to global health.[1] Optimising antimicrobial treatment strategies in patients offer an important direction to address this challenge. Infections are currently commonly treated using standard empirical protocols, which are designed to obtain broad pathogen coverage.[2,3] Such one-fits-all treatments neglect differences between patients and do not regard the causative pathogens. Moreover, there is a lack of consideration of the dynamic nature of patient physiological conditions and bacterial pathogens evolution within patients. Patients and pathogen characteristics can change during the course of an antimicrobial treatment due to for instance changes in organ function or development of AMR. Insufficient consideration of variability between pathogens and between- or within patients can lead to substantial variation in outcomes of standardised treatments, thereby contributing to the risk of treatment failure.[4] Strategies to further optimise antimicrobial treatments for individual patients and causative pathogens are therefore warranted.

The outcome of an infection is not ruled by one single factor, but rather by the interplay between the drug, the pathogen, and the patient, i.e. the host. To this end, the development of optimised and individualised antimicrobial treatment strategies requires a multifaceted approach where these factors, and the interactions, are considered.[5] It is imperative to obtain a quantitative understanding of the drug exposure, i.e. pharmacokinetics (PK), and how this relates to the dynamics of drug effect on pathogens, i.e. pharmacodynamics (PD), and how the host immune response relates to these PK-PD relationships. To this end, this thesis covers three main research themes: antimicrobial PK, bacterial evolution and antimicrobial PD, and host response to bacterial infections.

In order to derive optimised dosing strategies informed by knowledge of drug, host and pathogen interactions, quantitative pharmacological modelling approaches are essential. Several different modelling techniques are of relevance, depending on the specific goals and data availability. Data driven top-down approaches allow for empirical description of the data. Here, nonlinear mixed effects modelling (NLME) or population modelling is commonly used to fit observation-time profiles to characterise processes in patient data. Such population modelling approaches are valuable to quantify inter-individual variability within the population and to identify patient-specific predictors which may be used to individualise drug treatment. NLME modelling can also be applied to develop semi-mechanistic models, which allow incorporation of different degrees of mechanistic knowledge.[6] Finally, bottom-up mechanistic modelling approaches are constructed fully based on prior knowledge of biological and systems-specific properties. Such modelling approaches are relevant to explore hypothesis or generate predictions about scenarios where

data are not available. In this thesis a range of modelling techniques are used to contribute to the development of a quantitative understanding of the drug, pathogen, and host interactions that can be used to optimised dosing strategies.

Antimicrobial pharmacokinetics

Pharmacokinetics describes the relationship between drug dose and its concertation-time profile in the body. Typically, the PK is characterised for the total drug concentration, i.e. the sum of the free concentration and the concentration bound to proteins in the plasma. Total drug concentrations in plasma are still commonly used to inform dosing strategies of antimicrobials. However, it is the concentration of unbound drug at the site of infection that drives the antimicrobial effect.[7] When designing treatments, plasma protein binding and infection site concentration should be considered to ensure adequate efficacy. The antimicrobial concentrations in certain tissues, e.g. lung, may greatly differ from the plasma concentration.[8] Neglecting such discrepancies when designing dosing regimens may lead to suboptimal antimicrobial exposure at the infection site. Additionally, pathophysiological changes can lead to altered plasma and tissue-specific PK in critically ill patients compared to healthy volunteers.

To enable design of optimised antimicrobial dosing schedules, i.e. how much and how often the antimicrobial should be dosed, it is important to quantitatively characterise the drug exposure. NLME modelling is used to fit concentration-time data to quantify drug absorption, distribution, metabolism, and excretion in a population of individuals.[9] Such PK models are constructed using empirical compartments and generally describe plasma drug disposition. Due to the empirical nature of these PK models, they are not well suited for predicting tissue concentrations. Instead, tissue concentrations can be more effectively predicted using physiologically based PK (PBPK) models.[10] PBPK models are constructed using a bottom-up approach incorporating known physiology using system-specific parameters, which are combined with drug-specific parameters to predict tissue-specific PK. Altering system-specific parameters allows to investigate how changes in the biological system affect PK, allowing for example translating PK in healthy individuals to a patients through the incorporation of pathophysiological changes. PBPK models thus represent a valuable modelling approach to predict infection site PK in patients.

Bacterial evolution and antimicrobial pharmacodynamics

Pharmacodynamics (PD) describes the relationship between drug concentration and drug effects, which include both desired, i.e. efficacy, and undesired effects, i.e. toxicity. Antimicrobials drug effects are dynamic as they act on growing and evolving bacterial populations. Antimicrobials primarily act by either disrupting bacterial cell replication or inducing cell death. At the same time, bacterial pathogens exposed to antimicrobial can evolve AMR through mutation and selection.[11] In order to effectively predict antimicrobial treatment response, characterisation of the direct drug effects

is required as well as the adaptive response of bacterial pathogens. In practice, this is rarely considered during the design of antimicrobial dosing schedules.

Current approaches to guide and design antimicrobial treatment schedules relies on summary metrics, such as the minimum inhibitory concentration (MIC). The MIC is the most commonly used metric to quantify the antimicrobial susceptibility of bacteria, and represent the concentration that inhibits visible growth after a standardised incubation time. Although the MIC is a clinically relevant metric, it is derived for only a single time point, typically after 20 hours, while underlying antimicrobial PK-PD and infection in a patient are inherently dynamic. Shifting towards a more in-depth quantitative characterisation of the dynamic interaction between antimicrobials and bacterial pathogens could aid in improving treatments and supress AMR development.

Bacterial resistance evolution is typically studied with *in vitro* experiments using static or stepwise increasing antimicrobial concentrations. Such experiments can contribute valuable insight into antimicrobial PD, but also on the bacterial population dynamics of AMR evolution. However, these experiments fail to capture the effects of the fluctuating antimicrobial concentrations observed in patients, i.e. antimicrobial PK. How such differences in antimicrobial exposure might affect the resistance development the experiments are designed to study is not well understood. Another important aspect are evolutionary trade-offs of AMR. In some situations, the development of AMR can lead to evolutionary trade-offs, which increase antimicrobial susceptibility to other antimicrobials.[12] This phenomenon is called collateral sensitivity (CS). Exploiting CS could be of interest to counter the resistance development. However, the rational design of treatments that use the phenomenon of CS remains challenging.

Host immune response to bacterial infections

During a bacterial infection, the host's immune response is orchestrated to attack invading pathogens. Key to this immune response is the infection-induced production of host-associated inflammatory biomarkers, such as cytokines and other inflammatory proteins e.g. C-reactive protein (CRP) and procalcitonin (PCT). These markers may carry important insight into the current state of an ongoing infection, which potentially could be utilised to guide antimicrobial therapy. Both CRP and PCT are currently used clinically as markers for severe bacterial infections. PCT-based treatment algorithms to guide antibiotic de-escalation have been shown to reduce mortality and unnecessary antimicrobial exposure in septic patients.[14] Nonetheless, the prognostic value of these markers is still not fully established and their potential to further guide antimicrobial treatment remains to be established.[13] Biomarker based treatment monitoring could thus have the potential to support individualisation of antimicrobial therapy. However, for a biomarker to have a potential as a treatment-response biomarker its dynamics needs to be quantitatively characterised and shown to closely follow the infection. Currently,

such well-characterised biomarkers with suitable properties are lacking.

Simultaneously considering multiple immune biomarkers might provide more insight into the infection than one single marker.[15] However, the host response to invading bacterial pathogens is complex and not yet well understood. Characterising the correlations between biomarkers dynamics could aid in uncovering important interactions. Moving beyond correlations and simultaneous consideration of the known interactions of key components of the immune response could further help to unravel parts of the underlying immune mechanisms to infections. A better understanding of these mechanisms may aid in finding effective treatments for infection related immune dysregulation, which is driving the progression sepsis.

Integrating the dynamics of relevant host response biomarkers into model based treatment strategies allows for individualised treatment monitoring and adaptation. Top-down ordinary differential equation based modelling has been applied to describe immune biomarker dynamics in several animal species.[16–18] Using a similar approach to human biomarker data would allow for a quantitative characterisation, which is currently lacking. Although such models are data driven, they could potentially include some mechanistic components. However, they are often limited to only characterising a part of the immune response. A more holistic description of the immune response could be obtain through the use of systems biology or quantitative systems pharmacology models.[6,19] Such models are constructed using a bottom-up approach, requiring highly specific quantitative data to populate the model. Currently, the lack of data availability is impeding the development of such models. A qualitative modelling approach, such as Boolean modelling, could help to overcome the need of quantitative data while still providing a systems level characterisation of the immune response to bacterial infections. [20]

Thesis outline and scope

Section I: General introduction and outline

Section I outlines the standard antimicrobial therapy and the need for updated treatment strategies. In this section, three different research themes are defined; antimicrobial PK, bacterial evolution and antimicrobial PD, and host response to bacterial infections. Building on these research themes, and addressing their current shortcomings, the work in this thesis aimed to move toward the development of novel model-based treatment strategies to optimise and individualise antimicrobial therapy with explicit consideration of AMR development.

Section II: Pharmacokinetics of antimicrobials

Antimicrobial PK is the focus of *Section II*, with specific attention on the exposure

driving the antimicrobial effect, i.e. unbound drug at the site of infection. In **Chapter 2**, we characterised the total and unbound PK of teicoplanin in critically ill children through the use of NLME modelling (**Chapter 2**). This chapter aimed to identify predictors of protein binding and teicoplanin clearance to guide teicoplanin treatment in this vulnerable paediatric population. The following two chapters zoom in on pulmonary PK. **Chapter 3** describes the validation of a model predicting lung penetration ratio of anti-infective agents based on quantitative structural-property relationships (QSPR). This validation aimed to evaluate the predictive performance of the model relating to new classes of anti-infective agents. An adapted version of the QSPR-based model was incorporated into the lung PBPK modelling framework developed in **Chapter 4**. This chapter aimed to investigate the performance of three alternative modelling approaches, which allow for prediction of lung specific PK in situations with different data availability. The framework was also used to increase the understanding of how drug properties and pathophysiology affect pulmonary PK.

Section III: Bacterial evolution and antimicrobial pharmaco-dynamics

Section III is focussed on bacterial evolution and antimicrobial PD. **Chapter 5** aimed to elucidate how, and if, the choice of experimental system impacts bacterial evolutionary trajectories. This was evaluated in *Klebsiella pneumoniae* exposed to colistin in three different *in vitro* systems. Building on a separate set of *in vitro* time-kill experiments with *K. pneumoniae,* a semi-mechanistic model was developed (**Chapter 6**). This model was used to assess molecular mechanisms of resistance to β-lactam antibiotics and β-lactamase-inhibitors with the aim to identify the most important treatment targets to overcome resistance. Another strategy to suppress AMR development was investigated in **Chapter 7**, where the potential of exploiting evolutionary trade-offs in form of CS was evaluated. Here, we derived fundamental design principles of CS-based treatments using theoretical simulations. Although CS-based drug cycling regimens have been suggested previously, no clinical CS-based regimen has been developed. A mathematical framework was developed, which allowed for simulation of bacterial growth, kill, and resistance development during antimicrobial combination treatments. This approach permitted for the disentanglement of the impact of bacterial properties, such as CS and growth rate, on resistance development. In **Chapter 8**, the framework was applied to scenarios informed by experimental data of *Streptococcus pneumoniae.* This chapter aimed to evaluate the potential of combination treatments in *S. pneumoniae* and to identify which factors are driving resistance to fluoroquinolones.

Section IV: Host response to bacterial infections

Section IV includes four chapters covering the host response to bacterial infections and the possible use for host response biomarkers for antimicrobial

treatment individualisation. In **Chapter 9**, the current state of biomarker-based treatment individualisation is discussed. We also discuss how mathematical modelling can aid in advancing such approaches, which was demonstrated with an analysis using PCT in septic patients as proof-of-concept. However, the high variability relating to treatment and infection make the characterisation of host response biomarker dynamics challenging in patients. As a step towards a quantitative understanding of the host response to bacterial infections, the Toll-like receptor 4 mediated pathway was studied in healthy volunteers (**Chapter 10 and 11**)**.** Studying a selected part of the host response in healthy volunteers reduce variability compared to patients and provide a clear, although limited, picture of the response. Specifically, in **Chapter 10**, the dynamics of a novel host response biomarker, presepsin, in response to lipopolysaccharide (LPS) are reported. This chapter aimed to characterise presepsin dynamics and how it relates to other more established markers to assess presepsin potential as a treatment response biomarker. The dynamics of such markers needs to be quantitatively characterised to further enable biomarker-based treatment monitoring. **Chapter 11** aimed to quantitatively describe the dynamics of established host response biomarker in healthy volunteers by developing a set of mathematical models. To further understand the host response to LPS, a Boolean network model was developed in **Chapter 12**. This model provideed insight into the complex immune responds mediated by the Toll-like receptor 4, which plays a major role during sepsis. This chapter aimed to explore possible treatment targets in sepsis and potential treatment response biomarkers.

Section V: Summary, conclusions, and perspectives

In *Section V*, the results generated in previous sections are summarised and discussed (**Chapter 13**). Furthermore, this section outlines the future perspective on how to improve antimicrobial treatments on the basis of the three research themes of antimicrobial PK, bacterial evolution and antimicrobial PD, and host response to bacterial infections.

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