Improving antimicrobial therapy for Buruli ulcer
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Chapter 1

Introduction and outline of the thesis

“The neglected tropical diseases provide another example of our solidarity. These diseases do not travel internationally, threaten the health or economies of wealthy countries, or make headline news. Yet they cause immense suffering and disability for millions of people and anchor them in poverty.”

Dr. Margaret Chan,
Address to WHO staff, 4 January 2007.
Buruli ulcer (BU) is a skin and subcutaneous tissue infection caused by *Mycobacterium ulcerans*. The disease usually manifests as single lesion on the extremities of patients, most of which are under the age of 15 years. The nodule, ulcer, oedematous lesion and plaque are the four recognized forms of BU (1). BU is one of the 21 neglected tropical diseases (NTDs), as defined by the World Health Organization. These diseases affect more than 1 billion people globally, yet are neglected and underfunded in disease control and research as they mainly occur in poor, rural, and marginalized communities in subtropical and tropical regions (2). The chronic and non-lethal nature of many NTDs further prevents them from being addressed on a global scale; low mortality does not create the urgency for public attention compared to diseases like malaria or Ebola (3). Patients with NTDs suffer from inequity as many cannot access appropriate treatment and are stigmatized, and discriminated against. Basic human rights are hence infringed upon frequently (4).

Neglected tropical disease are highly diverse in their epidemiology, pathobiology and transmission. The importance of research and public health efforts to reduce the burden of NTDs has been acknowledged by their inclusion in the United Nations Sustainable Development Goal (SDGs) in 2013 with a pledge to “leaving no one behind” (5). Specifically, goal 3.3 calls for the reduction of the burden of HIV/AIDS, tuberculosis, malaria, neglected tropical diseases and priority non-communicable diseases (6). Hence they are interrelated with many other SDG goals, such as universal health coverage or clean water and sanitation and reducing the burden of NTDs is thought to have a positive outcome on many other indicators (7).

The main strategies of the global public health community to address NTDs were laid out in resolution WHA 66.12 adopted at the World Health Assembly 2013 (8) and the 2012 WHO roadmap for NTDs (2): Preventive chemotherapy and transmission control (PCT), innovative and intensified disease management (IDM), vector ecology and management, control of neglected zoonotic diseases and improvement of water, sanitation and hygiene (WASH). BU, due to its complex mechanism of transmission and slow disease progression, is targeted for control through IDM. Diseases like BU, for which no preventive chemotherapy is available and the cause is an environmentally present pathogen that cannot be eradicated, need special attention. The WHO roadmap set ambitious goals to improve the situation for NTDs. Aims for BU were completion of a clinical study on oral antimicrobial therapy and implementation of the results into control and treatment by 2015, and curing 70% of occurring cases with antibiotics by 2020 (8). BU mainly occurs in rural areas and cases are thus not easily recognised by health authorities. Lack of local and communal resources further hinders

A BRIEF INTRODUCTION TO THE HISTORY OF BURULI ULCER

“The right leg, from above the knee, became deformed with inflammation and remained for a month in this unaccountable state, giving intense pain, which was relieved temporarily by deep incision and copious discharge. (…) my strength was prostrated; the knee stiff and alarmingly bent, and walking was impracticable.”

J. A. Grant, A walk across Africa or domestic scenes from my Nile journal; William Blackwood and sons, Edinburgh and London, 1864. The first probable description of the Buruli ulcer.

BU is a fairly recently described infectious disease. The first report of an ulcerative condition attributed to BU is by James Augustus Grant in his book entitled “A walk across Africa or domestic scenes from my Nile journal” from 1864 (11). On a quest to discover the origin of the White Nile, Grant described an inflammatory, discharging lesion on his right leg while in Congo (quote above). After several incisions, eventually Grant’s lesion healed, enabling him to continue his journey, even though he suffered from wound contracture (11). Both the natural history with slow healing and impaired range of motion as sequelae suggest it to be the first description of BU (12). Another early report of ulcers nowadays attributed to BU was put forward by Sir Albert Ruskin Cook, a British missionary doctor. He established the Mengo Hospital in Uganda in 1897 (13). There, he noted an accumulation of cases of an at the time unknown ulcerative skin disease, which is now believed to be BU (14).

The first description of *Mycobacterium ulcerans* as the causative agent of BU was published in 1948 (15). MacCallum et al found acid-fast bacilli in tissue specimens from six patients with an unusual skin ulceration from Bairnsdale, Victoria, Australia, at first attributed to M. tuberculosis. However, this hypothesis was refuted by the observation of distinct histopathological characteristics that did not resemble TB as well as the inability to culture the organism at 37°C. Serendipitously, cultures kept in an incubator with defective heat circulation resulting in a drop of temperature to 33°C were successful and yielded the first laboratory grown *M. ulcerans*. By establishing the ideal culture conditions, the organism could soon be grown directly from patient samples (15). In the 1950s and 1960s reports accumulated describing incident cases in West Africa. Several hundreds of cases were re-
ported from Congo and Uganda, namely Buruli county, after which the disease was finally named (16-18). The increasing public health interest and discovery of the etiologic agent facilitated modelling the disease in the laboratory. Fenner and colleagues eventually developed the mouse footpad model of BU and the disease has since been studied in this and other pre-clinical models (19).

**CONCEPTS OF MYCOBACTERIUM ULCERANS PATHOBIOLOGY**

*M. ulcerans* is closely related to *Mycobacterium marinum*, an aquatic bacterium causing skin sores in fish and humans; in fact, the two bacteria share >98% nucleotide sequence identity (20). Genome comparison studies have demonstrated great homology between geographically distinct *M. ulcerans* isolates suggesting that little gene transfer and recombination occur. Two unique genetic characteristics set *M. ulcerans* apart from *M. marinum*: the presence of the pMUM001 plasmid and over 200 copies of the insertion sequence IS2404 (20). The current evolutionary scenario holds that *M. ulcerans* acquired pMUM001 from an unknown source through horizontal gene transfer. This giant 174 kb megaplasmid encodes for several polyketide synthases (PKS) that produce mycolactone, the main pathogenic toxin of *M. ulcerans* (21). Experimental deletion and inactivation of the plasmid renders it avirulent (22). The vast abundance of IS2404 within the *M. ulcerans* genome disrupts many promoter regions and coding sequences (23). The *M. marinum* genome measures 6.6 Mb, whereas the *M. ulcerans* genome is 5.8 Mb and reductive evolution adapting to a new ecological niche it thought to account for this difference (23). This reductive evolution is thought to have in which there was no evolutionary advantage for functionality of such genes (20). Three lineages of mycolactone producing mycobacteria are recognized (12). The ancestral lineages that are believed to have evolved from *M. marinum* about 400,000 years ago are lineage 1 and 2. Lineage 1 has been found in human patients in South America, Asia and Mexico but also comprises frog pathogen variants. Genetically distinct clones from Japan are described as lineage 2. Lineage 3 is comprised of genotypes observed to cause both human and zoonotic disease in Africa, Australia and South East Asia, Papua New Guinea, in particular (12).

Mycolactone, the toxin produced by the PKS encoded by the pMUM001 plasmid, consist of a heterogeneous poly-unsaturated southern chain and a conserved macrolactone ring and north chainern and is produced close to the bacterial membrane. Different mycolactones, A/B, C, D, S1, S2 and E that vary in virulence have been described and attributed to *M. ulcerans* (12). Mycolactone causes cytotoxicity, immunosuppression and analgesia (1,24).
Mycolactone targets bacterial scaffolding proteins leading to instability and cell death and it inhibits translocation of important proteins through the endoplasmic reticulum impairing bacterial metabolism (25). The endoplasmic reticulum membrane protein Sec61 has specifically been associated with M. ulcerans pathobiology. Inhibition of Sec61 leads to both cytotoxicity (26) and immunomodulation (27).

As its close relatedness to the aquatic M. marinum suggests, M. ulcerans has been associated with water bodies; stagnant and swammy areas but also unnatural disruption of water bodies such as the building of dams has been linked to outbreaks (28). If environmentally present M. ulcerans breaches the skin barrier, the disease is contracted, as pre-clinical studies suggest (29). In Australia, mosquitoes have been suggested be vectors propagating the pathogen (29,30), whereas in other regions aquatic insects have been suggested to be implicated in the transmission (31). The transmission may be diverse and vary by geographic setting, depending on the local ecology but also socio-demographic determinants and human behavior (1).

**SCOPE OF THIS THESIS**

This PhD thesis focuses on the BU WHO priority research item 3: Drug treatment and new treatment modalities. Its main body consists of laboratory studies, enriched by literature review and engagement with and discussion of the current knowledge, as well as analysis of public health data. This introduction, Chapter 1, follows a description of Buruli ulcer epidemiology. Then, current knowledge on antimicrobial therapy of BU is reviewed. The experimental part of this thesis consists of five manuscripts, describing histopathology and immunology of the disease in the mouse model and the pre-clinical evaluation and refinement of antimicrobial regimens to treat BU, before concluding with a summary pointing out strategies for the way ahead.

As an introduction to BU worldwide, Chapter 2 explores epidemiological data reported to WHO from 2010 – 2017 and maps the global epidemiology of the disease. Programmatic targets for disease control set by WHO are analysed and the global progress on these programmatic targets is stated.
Chapter 3 narrates the translation prior research into the paradigm change from surgery to antimicrobial drug therapy in Buruli ulcer. Subsequently, Chapter 4 is a comprehensive review of the antimicrobial treatment of *M. ulcerans*. The susceptibility of the bacterium to antimicrobials is discussed, as are relevant animal model studies and clinical trials.

Chapter 5 seeks to refine the currently used pre-clinical *M. ulcerans* infection model by application of modern *in vivo* imaging (IVIS) technology. Visualisation of light-emitting bacteria allows for both bacterial quantity estimation as well as localisation of the pathogen within the living host. It also describes and discusses the histopathology and immunology related to infection with an autoluminescent *M. ulcerans* reporter strain.

New treatment modalities for BU are direly needed. As reports in the literature suggested, avermectins, natural products derived from *Streptomyces* species kill the related *M. tuberculosis* *in-vitro*. Chapter 6 explored and demonstrated an antimicrobial effect of the avermectin ivermectin *in vitro*. Chapter 7 consists of two pre-clinical studies in mice; first, the pharmacokinetics of repeated, high-dose ivermectin was evaluated to identify a dosing regimen to achieve plasma concentrations exceeding the minimum inhibitory concentrations *in vitro*. Secondly, the anti-mycobacterial effects of ivermectin and selamectin were tested *in vivo*.

BU currently is treated with rifampin plus either clarithromycin or streptomycin. However, the dose of rifampin is based on outdated cost-efficacy and side-effect arguments. Higher doses of rifampin are affordable and have been shown to be safe, in an effort to promote high-dose rifampin therapy for tuberculosis. In Chapter 8 we performed pre-clinical dose-ranging of rifampin and rifapentine in *M. ulcerans* infected mice.

Rifampin is a backbone drug in the treatment of BU, it is bactericidal, available and highly efficient in the treatment of BU. However, the currently proposed companion macrolide clarithromycin only exerts bacteriostatic effects, and (bi-directional) drug-drug interactions might render the rifampin-clarithromycin combination less efficient. In order to find an alternative companion drug to rifampin, oxazolidinones were investigated in Chapter 9. Sutezolid and tedizolid are modern oxazolidinones that may be toxic than linezolid and were non-inferior to clarithromycin.

Chapter 10 summarizes the results of the abovementioned pre-clinical studies on new or improved antimicrobial regimens for BU.
Chapter 11 explores the future of BU therapy highlighting how highly efficient, short-course regimens can be key to BU disease control and drafts a proposal for future research and integration with other neglected tropical skin diseases.
LITERATURE


