Lantibiotics as prospective antimycobacterial agents

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Mycobacteria contribute substantially to human infectious disease, most notably the primary pathogens of tuberculosis (TB), leprosy and Buruli ulcer. Global prospects for TB control are challenged by the emergence of drug-resistant strains. If we are to stay ahead of mycobacterial strains developing resistance to chemotherapeutic regimens, all new agent leads must be explored. Lantibiotics, either native or bio-engineered, represent one such avenue to enhance our artillery against drug-resistant strains.

Bacteria of the genus *Mycobacterium* are grouped among the actinomycetes and include non pathogens, opportunistic pathogens and highly pathogenic species of both humans and animals. The mycobacteria cause more human disease, including, most notably, tuberculosis (*Mycobacterium tuberculosis* and *Mycobacterium bovis*), leprosy (*Mycobacterium leprae*) and Buruli ulcer (*Mycobacterium ulcerans*) than any other bacterial genus. *Mycobacterium tuberculosis*, the etiological agent of TB, is responsible for approximately 9.5 million new cases per year developing active disease. This can result in 2–3 million deaths.¹ While *M. tuberculosis* is human-specific, the closely related *Mycobacterium bovis* can infect both humans and animals and lead to TB regardless of the host. Another *Mycobacterium* of zoonotic interest is the multihost (cattle, sheep, goats and camelids *inter alia*) chronic enteric pathogen *Mycobacterium avium* subsp. *paratuberculosis* (Map) which causes Johne disease in ruminants. Map has been implicated as one cause of human Crohn disease, although evidence is equivocal.² However, the bacterium has been recovered from food products derived from cattle and has been shown to be resistant

to many chemical processes. The majority of the *Mycobacterium* species, often referred to as non-tuberculosis mycobacteria (NTM) can be potentially pathogenic to humans or animals. The majority of these species are natural inhabitants of various aquatic and terrestrial environments and many have been shown to have clinical relevance.³ Clinically relevant species include *Mycobacterium avium* [and other members of the *M. avium* complex (MAC)], *Mycobacterium kansasii*, *Mycobacterium marinum*, *Mycobacterium xenopi* and *Mycobacterium abscessus*. They may cause disease or infection in both immunocompetent and immunocompromized patients. Clinical symptoms can manifest as: pulmonary disease, hypersensitivity pneumonitis, (cervical) lymphadenitis and cutaneous diseases. Although these mycobacterial species are not considered as obligate human pathogens, they nevertheless can be considered a health risk particularly to immunocompromized patients.

Treatment of the major mycobacterial human pathogens involves multi-drug or combination therapies. The World Health Organisation (WHO) recommended first-line treatment of *M. ulcerans* (Buruli ulcer) is an eight week treatment with rifampicin and streptomycin/amikacin while leprosy patients are treated with a combination of rifampicin, clofazimine and dapsone. Currently, active TB is treated by combination therapies that consist of three or more drugs (most typically four) selected from known anti-TB agents. Directly observed treatment, short course (DOTS) is the treatment approach recommended by the WHO. This includes two months administration of isoniazid (INH), rifampicin (RIF), pyrazinamide

Key words: lantibiotics, mycobacteria, resistant, antimycobacterial, bacteriocins

Submitted: 10/04/10

Accepted: 10/04/10

Previously published online:
www.landesbioscience.com/journals/
biobugs/article/13855

DOI: 10.4161/bbug.1.6.13855

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(PZA) and ethambutol (EMB) (the intensive phase), followed by INH and RIF for an additional 4 months (the continuation phase). Even though multi-drug therapy for treatment of TB has been the strategy advocated, drug-resistant *M. tuberculosis* strains have emerged. Strains of *M. tuberculosis* that are resistant to both INH and RIF, with or without resistance to other drugs are termed multi-drug resistant (MDR-TB). They require a further 2 years of treatment with second-line drugs, such as (fluoro)quinolones, aminoglycosides, ethionamide, D-cycloserine or basic peptides.⁴ Recently, the emergence of extensively drug-resistant TB (XDR-TB), caused by MDR-strains also resistant to two major second-line agents (aminoglycoside and fluoro quinolone), has been alarming.^{5,6} There is also much concern that the TB situation will worsen with the growing human immunodeficiency virus (HIV) pandemic worldwide, as the viral infection can weaken the host immune system. Drug-resistant TB arises following a spontaneous mutation in the genome of the organism and largely occurs as a result of inappropriate prescribing, poor treatment adherence, irregular drug supply or poor drug quality.

Rifampicin was the last new drug to be incorporated in the current standard anti-TB regimen. This was introduced almost 40 years ago. Since then, few new agents for TB have been developed because of the lack of market opportunities.⁷ However, in recent years increased investment in the area of TB therapeutics has been witnessed in an effort to tackle this urgent, global, public health need. Developments have mostly centred on (a) existing anti-TB agents (b) known antibiotic classes not yet approved for TB treatment and (c) novel chemical series. Among the classes of novel chemical series being explored are nitromidazoles, diaryl quinolines, pyrroles, macrolides and oxazolidinones.⁷ In the quest for novel chemotherapeutic agents, to combat mycobacterial and other human diseases, no leads can be ignored.

The current need for anti-mycobacterial agents with novel target molecules has coincided with advances in technical approaches for the structural and functional analysis of lantibiotics, which are ribosomally synthesized peptides

produced by Gram positive bacteria. The potential use of lantibiotics in food, human and animal applications has been well documented,^{8,9} however given the emergence of antibiotic resistance across many bacterial genera, lantibiotics demonstrate an alternative to existing chemotherapeutic agents.

To date, more than 50 different lantibiotics have been identified with different structures and modes of action. Nisin A and lactacin 3147, produced by *Lactococcus lactis*, are the best characterised lantibiotics. While nisin is active as a single moiety, lactacin 3147 requires two peptides for optimal activity. These and other lantibiotics exert their antimicrobial effect through more than one mode of action, namely by either forming pores in the target cell membrane or inhibiting cell wall synthesis.⁹ Although it may be expected that the unique cell wall structure of mycobacteria would challenge such mechanisms of action, previous studies have demonstrated antimycobacterial activity of lantibiotics/bacteriocins against mycobacterial species.¹⁰⁻¹² Also, their bacteriocidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci and oxacillin-resistant Gram positive bacteria has been demonstrated¹³ and many new chemical applications of a variety of lantibiotics are currently being pursued. Lantibiotics have several features which advances their candidacy as therapeutic agents: stability and cost effective production processes, an auto-regulation system, they can be produced by food-grade bacteria and have a relatively broad killing spectrum. Furthermore, with the increased knowledge of bacterial genetic organisation and biosynthetic pathways, the potency of lantibiotics as antimicrobial agents can be enhanced through genetic engineering viz a viz mutations, gene fusion or manipulation of the producing strain.¹⁴ There also exists the possibility of using lantibiotics as adjunct therapies in conjunction with traditional chemotherapeutic agents. While the prospect exists for 'designer lantibiotics' to meet the challenges of drug-resistant mycobacterial diseases, it must be acknowledged that such agents must be active against the intended target

pathogen and stable and safe in the proposed environment of use.

In the paper presented previously by Carroll et al. the bacteriostatic activity of bio-engineered versions of the lantibiotic nisin was demonstrated against clinically important mycobacterial species. This study affirmed work of others that lantibiotics can possess antimycobacterial properties and furthermore demonstrated improved antagonistic activity of the bio-engineered derivatives over the native nisin A. Different potencies were observed between pathogenic mycobacteria in the study, emphasising the possible requirement for species-specific designer lantibiotics in the future. However, it should be noted that the bacteriostatic activity was demonstrated against proliferating cells. A further challenge exists for lantibiotics to display bactericidal activity and anti-mycobacterial dissonance against latent/dormant forms of the bacteria.

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