Exploring prospects of novel drugs for tuberculosis

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Abstract: Tuberculosis remains a disease with an enormous impact on public health worldwide. With the continuously increasing epidemic of drug-resistant tuberculosis, new drugs are desperately needed. However, even for the treatment of drug-sensitive tuberculosis, new drugs are required to shorten the treatment duration and thereby prevent development of drug resistance. Within the past ten years, major advances in tuberculosis drug research have been made, leading to a considerable number of antimycobacterial compounds which are now in the pipeline. Here we discuss a number of these novel promising tuberculosis drugs, as well as the discovery of two new potential drug targets for the development of novel effective drugs to curb the tuberculosis pandemic, ie, the coronin 1 and protein kinase G pathways. Protein kinase G is secreted by mycobacteria and is responsible for blocking lysosomal delivery within the macrophage. Coronin 1 is responsible for activating the phosphatase, calcineurin, and thereby preventing phagosome-lysosome fusion within the macrophage. Blocking these two pathways may lead to rapid killing of mycobacteria.

Keywords: tuberculosis, treatment, drug-resistance, drug targets

Introduction

Mycobacterium tuberculosis continues to be one of the world's most debilitating and deadly pathogens. Tuberculosis accounted for an estimated 1.7 million deaths in 2009, and the incidence is higher than ever before, with 9.4 million new cases.¹ Efficacious drugs exist, but their success in treatment depends on rigid implementation of therapy, access to treatment, and adherence over a considerable time span. This has consequences regarding the logistics of tuberculosis control programs, which currently fail in many settings in the view of the human immunodeficiency virus (HIV)/acquired immune deficiency syndrome copandemic. There are still sizeable populations, especially in sub-Saharan Africa, which have no access to tuberculosis control through the Directly Observed Treatment Short course (DOTS) strategy of the World Health Organization. In addition to the urgent need for novel drugs, DOTS coverage should be increased further to reduce ongoing transmission.²

Although tuberculosis drug research over the past 10 years has led to the development of a few novel agents which are currently in different stages of clinical evaluation, the preceding 30 years had been painfully silent in the field of tuberculosis drug research. The rise in drug resistance among *M. tuberculosis* strains has become a severe threat to public health on a global scale. With an estimated 440,000 cases of multidrug-resistant tuberculosis (defined as *Mycobacterium tuberculosis* resistance to at least rifampicin and isoniazid) and extensively drug-resistant tuberculosis (defined as multidrug-resistant

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tuberculosis plus resistance to a fluoroquinolone and at least one second-line injectable agent, ie, amikacin, kanamycin, and/or capreomycin) now being reported in 58 different countries, the epidemic is ever increasing.³

Although mortality rates for extensively drug-resistant tuberculosis have now been reduced from the initially reported nearly 100%⁴ to approximately 45%,⁵ it remains a challenge to treat infected individuals because of the long treatment duration required and inevitable usage of substances of high cost and with unfavorable safety profiles. In HIV-positive patients in Southern Africa, at least 50% of all adults have been documented as suffering from tuberculosis.⁶ When looking at coinfections in confirmed tuberculosis cases with HIV, these reached up to 95% in the Johannesburg setting, which is the highest coinfection rate ever described to date in the literature.⁷ All this puts additional pressure on the need to develop more effective strategies to curb the tuberculosis pandemic, and in particular, this includes novel, more effective, and well tolerated drugs.

Apart from the need for new drugs to treat drug-resistant tuberculosis, several challenges are faced, even for drug-susceptible tuberculosis. Drug-susceptible tuberculosis still needs to be treated with a regimen containing at least four different drugs, and treatment should be continued for at least 6 months. Compliance and adherence would increase with shorter treatment courses containing fewer drugs. Furthermore, there are important interactions with the rifamycins and the most widely used antiretroviral drugs for HIV, making coinfection with tuberculosis and HIV complicated to treat and creating a need for new drugs which lack these interactions. Short and safe treatment regimens for latent tuberculosis (with an estimated 2 billion people living with latent tuberculosis serving as a continuous reservoir for new active cases) still need to be developed.

In this review, we briefly outline the problem of multidrug-resistant and extensively drug-resistant tuberculosis, give an overview of novel regimens currently in clinical evaluation, describe the mechanisms of survival of *M. tuberculosis* in infected hosts, and propose avenues currently being addressed in the authors' laboratories that may contribute towards the development of therapies targeting drug-susceptible, multidrug-resistant, and extensively drug-resistant tuberculosis.

Multidrug and extensively drug-resistant tuberculosis

Control programs are often overburdened in highly endemic countries, giving rise to *M. tuberculosis* drug resistance due

to a range of predisposing factors and circumstances.⁸ The emergence of multidrug-resistant and extensively drug-resistant tuberculosis strains has been prominent in parts of the former USSR, particularly the Baltic republics and some Western megametropolitan areas such as New York City, as well as India, China, and the African continent, with a focus on its south, as highlighted by the first described outbreak of an extensively drug-resistant strain of *M. tuberculosis* in KwaZulu Natal, with excessive mortality.⁴

The mechanisms involved in the development of multidrug resistant and extensively drug-resistant tuberculosis are complex and determined by the mycobacterium, the host, and iatrogenic factors. Firstly, mycobacteria have a high degree of intrinsic resistance to most antibiotics and chemotherapeutics due to the low permeability of the mycobacterial cell wall.⁹ Numerous chromosomal mutations have been associated with the development of drug resistance in tuberculosis.¹⁰ One of several host factors predisposing to tuberculosis drug resistance is immunosuppression.¹¹ However, development of drug resistance against tuberculosis is mainly associated with intensive drug use and lack of compliance with treatment. Epidemics of drug-resistant tuberculosis, as for example in South Africa, can be largely attributed to poor performance of control programs and low cure rates over many years.¹²

Novel drugs to combat TB

The drugs currently used for tuberculosis were discovered before 1970. After that, the world of tuberculosis drug research remained silent for over 30 years. Over the past decade, drug development efforts have increased, fuelled by the upcoming threat of drug resistance and the tuberculosis epidemics which are ever increasing, and also influenced by the expansion of the HIV pandemic.

At the time of writing, there are currently at least 13 drugs in different stages of preclinical or clinical evaluation (<http://www.newtbdrugs.org/pipeline.php>) for the treatment of tuberculosis (see Table 1).^{5,13–26} They can be roughly divided into three categories, ie, novel drugs, drugs currently licensed for other indications but “repurposed” for tuberculosis, and the current first-line tuberculosis drugs which are re-evaluated to optimize their efficacy. There are two main lines of development, ie, improving treatment of drug-sensitive tuberculosis (cutting down on the required duration of therapy) to yield higher cure rates and curb development of resistance, and improving and shortening treatment of drug-resistant tuberculosis. In the authors' view, these should not be considered as competing but as complementary areas of

Table 1 Overview of antituberculous drugs currently in different stages of clinical development, the chemical class to which they belong, and their mechanism of action

Drug	Chemical class	Mechanism of action	Phase of clinical development
Novel drugs			
Bedaquiline (TMC207) ^{18,19,22} (Tibotec BVBA, Beerse, Belgium)	Diaryquinolones	PPI blocking mycobacterial ATP synthase	II, III
SQ109 ²³ (Sequella, NIH, Rockville, MD)	Ethylenediamine	Inhibition of cell wall synthesis	II
Delamanid (OPC-67683) ^{13,21,25} (Otsuka Pharmaceuticals Inc, Tokyo, Japan)	Nitroimidazoles	Inhibition of cell wall mycolic acid biosynthesis	III
PA-824 ²⁰ (TB Alliance, New York, NY)	Nitroimidazoles	Inhibition of cell wall mycolic acid biosynthesis	II
Licensed drugs being repurposed for use in tuberculosis			
Moxifloxacin ^{5,24,26}	Fluoroquinolones	Inhibition of DNA replication and transcription	III
Gatifloxacin ^{24,26}	Fluoroquinolones	Inhibition of DNA replication and transcription	III
Linezolid ^{15,16}	Oxalidinones	Inhibition of protein synthesis	II
Tuberculosis drugs re-evaluated to optimize efficacy			
Rifapentin ^{14,17}	Rifamycins	Inhibition of DNA-dependent RNA synthesis	II, III

Note: References mentioned are clinical trials reporting on efficacy, safety, and pharmacokinetics.

interest because both need to be addressed in order to turn the tide against tuberculosis at the “drug front”.

Although compared with ten years ago many promising new drugs are being developed, a further understanding of the pathogenesis of tuberculosis, latent infection, and development of drug resistance is needed in order to identify novel drug targets which may pave the way for further drug development.

Mechanisms of survival of *M. tuberculosis* within infected hosts

To be able to interfere with the persistence mechanisms developed by *M. tuberculosis*, it is important to understand the virulence mechanisms that the organism utilizes to establish itself within its human host. After entry into the host organism via the respiratory tract, mycobacteria are phagocytosed by macrophages and resist lysosomal degradation, thus allowing them to survive as well as to multiply within macrophage phagosomes, thereby circumventing the normal host response that would normally result in lysosomal degradation of the bacilli.^{27–29} Subsequently, *M. tuberculosis* uses an array of strategies to remain viable within an infected host, including escape into the cytosol,³⁰ residence outside macrophages, for example, within caseous regions of granulomas,^{31,32} modulation of nitric and oxygen stress³³ and regulation of autophagic processes.³⁴ In fact, *M. tuberculosis*, as one of the most successful pathogens known, has evolved an array of mechanisms to counteract the host immune response at virtually every imaginable level.³⁵

A comprehensive discussion of these mechanisms falls well beyond the scope of this paper, and the reader is referred to recent excellent review articles. Here, we focus on those mechanisms that *M. tuberculosis* has evolved to modulate intraphagosomal survival, and given the attenuation of *M. tuberculosis* strains that have lost the capacity to survive within macrophages,^{36,37} or even reside within the macrophage cytosol,³⁰ targeting intramacrophage survival mechanisms is likely to contribute to the control of *M. tuberculosis* proliferation.

Escape of mycobacteria from host defenses may also allow reactivation of tuberculosis in adults. This occurs when so-called dormant foci left in the host after a primary infection become reactivated.³⁸ Reactivation can occur when the host immune system fails to control bacterial growth or when the immune system is deteriorating, eg, as a result of malnutrition, overcrowding, or stress,³⁹ resulting in uncontrolled bacterial growth and death of the host.

Because of the important role of the macrophage phagosome as an escape site for *M. tuberculosis*, substantial efforts have been directed at defining the biology of mycobacterial entry and survival inside phagosomes. The mycobacterial phagosome, because it is derived from the plasma membrane, has many features in common with the membrane.^{40–43} However, in contrast with normal phagocytosis, in which phagosomal content is delivered to lysosomes either by maturation or through vesicular traffic of intermediate vesicles, mycobacteria actively resist lysosomal delivery.²⁷

Several strategies have been used to define mycobacterial virulence factors. These have included characterization of the *erp* gene product through generation of mutant strains,⁴⁴

proteins with a regulatory function, such as sigma factors as well as enzymes that function in different mycobacterial biochemical pathways, such as isocitrate lyase and glutamine synthetase.^{45–48} On the host side, the recently identified interferon-gamma-induced *LRG-47* gene was suggested to act as a vacuolar trafficking regulator necessary for the control of intracellular mycobacterial growth.⁴⁹

Different signaling pathways have been implicated in the survival mechanisms for pathogenic mycobacteria, including modulation of Ca^{2+} signaling upon entry⁵⁰ and regulation of phagosome-lysosome fusion through phosphorylation/dephosphorylation.⁵¹

Thus, *M. tuberculosis* uses multiple strategies to circumvent innate host immunity to infections. This unique capacity of *M. tuberculosis* to remain viable within the mycobacterial phagosome by avoiding lysosomal delivery within macrophages may be important for its capacity not only to survive for prolonged periods but also to cause severe disease and death in infected individuals.

Discovery of novel drug targets to block survival of *M. tuberculosis*

The capacity of *M. tuberculosis* to cause disease lies in its ability to avoid destruction within those cells that normally destroy all incoming bacteria, namely the macrophages. While bacteria are normally internalized into phagosomes from which they are transported to lysosomes where they are destroyed, *M. tuberculosis* actively blocks their transfer to lysosomes, allowing them to survive for prolonged times

within phagosomes. A long-term interest in the mechanisms utilized by *M. tuberculosis* to circumvent host resistance, thereby allowing these bacteria to proliferate and cause disease, has been maintained in the laboratories of RJ and JP.

Recent work has unraveled two pathways via which *M. tuberculosis* manages to survive within host cells. One mechanism relies on the secretion of a eukaryotic-like kinase by *M. tuberculosis*, ie, protein kinase G, the expression of which is essential to block lysosomal delivery.^{52,53} A second strategy relies on the retention of a host molecule, coronin 1, at the mycobacterial phagosome that is responsible for activating the phosphatase, calcineurin.^{54,55} Interestingly, current work suggests that targeting of protein kinase G as well as the coronin 1/calcineurin pathway may result in rapid killing of internalized mycobacteria. Whether or not inhibition of these recently discovered pathways would block survival and proliferation of multidrug-resistant and extensively drug-resistant strains of *M. tuberculosis* remains to be analyzed.

Targeting protein kinase G to promote killing of *M. tuberculosis*

Protein kinase G was discovered in a search for mycobacterial factors that promote survival of pathogenic mycobacteria within host macrophages. Protein kinase G is a eukaryotic-like serine/threonine protein kinase that is not required for mycobacterial growth per se but is essential for its survival within host macrophages (Figure 1), and has been discussed in detail elsewhere.^{53,56} Protein kinase G is one of the 11 serine/threonine protein kinases found in

Figure 1 Protein kinase G-mediated and coronin 1-mediated survival within host macrophages.

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M. tuberculosis, and the only soluble kinase maintained in the genome of *M. leprae* that is believed to have retained the minimal set of genes essential for virulence.⁵⁷ The presence of domains typically found in proteins from eukaryotic species as well as the finding that protein kinase G is dispensable for in vitro growth of *M. tuberculosis*^{56,58} suggests that protein kinase G has arrived in the *M. tuberculosis* genome through horizontal gene transfer and has been maintained as a virulence factor important for its survival inside the eukaryotic host.⁵⁹

Importantly, mycobacteria overexpressing a kinase-dead mutant of protein kinase G are rapidly transferred to lysosomes and killed, thus demonstrating that protein kinase G activity is crucial for mycobacterial survival. The fact that protein kinase G is translocated into the host cytosol suggests that compounds aimed at blocking protein kinase G activity do not require translocation across the only slightly permeable mycobacterial cell wall.⁵³

Together, these findings make protein kinase G an attractive and promising drug candidate. Indeed, blocking protein kinase G activity by a specific small molecular weight inhibitor, ie, the tetrahydrobenzothiophene, AX20017, results in rapid transfer of mycobacteria to lysosomes and killing of intracellularly residing bacilli.^{53,56} Furthermore, detailed biochemical analysis of the domains of protein kinase G has revealed several key residues that are crucial for both its in vitro kinase activity as well as the virulence function of protein kinase G within infected macrophages.^{60,61}

Two recently obtained sets of results have contributed to the validation of protein kinase G as a potential drug target. First, infecting mice with *M. tuberculosis* lacking protein kinase G resulted in dramatically prolonged survival of infected mice (mean survival time prolonged from 12 weeks to >50 weeks, unpublished data). This suggests that targeting protein kinase G in vivo may significantly alter the outcome of infection with *M. tuberculosis*. Second, we have recently obtained the x-ray structure of protein kinase G complexed with its inhibitor (Figure 2). The structure shows that protein kinase G contains a unique ATP-binding pocket that is different from any of the 491 human kinases known; in fact, a potent protein kinase G inhibitor showed virtually no activity when tested against a panel of 28 different kinases originating from the six major kinase groups. Thus, the structure of protein kinase G was revealed to be distinct from the host cell kinases, allowing its efficient targeting without blocking host cell kinases.⁵² Interestingly, docking studies using the tridimensional structure of protein kinase G identified potential novel inhibitors of the withanolide compound class.⁶²

Figure 2 Structure of protein kinase G with inhibitor.

Note: Inset: protein kinase G crystal.

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These preliminary findings suggest that targeting protein kinase G may enable inhibition of *M. tuberculosis* growth within host cells by allowing the macrophages to carry out their natural innate immune function, namely, shuttling *M. tuberculosis* to degradative lysosomes.

Inhibition of coronin 1-mediated signaling to block resistant *M. tuberculosis*

In 1999, coronin 1, also known as tryptophan aspartate-containing coat protein or p57,⁶³ was described as a protein that was actively retained in mycobacterial phagosomes, and was predicted to be involved in blocking the delivery of pathogenic mycobacteria to lysosomes (Figure 1).⁵⁴ This suggested an essential role for coronin 1 in protecting the mycobacterial phagosome from fusion with lysosomes,^{54,64} confirmed subsequently using siRNA-mediated knockdown of coronin 1.^{65–67} Moreover, mycobacteria are effectively destroyed within Kupffer cells, the major macrophages in the liver that do not express coronin 1.⁵⁴

How coronin 1 mediates the survival of pathogenic mycobacteria has been revealed recently by generating mice lacking coronin 1. It turns out that while mice lacking coronin 1 are perfectly healthy, coronin 1 is required for activation of the Ca^{2+} -dependent phosphatase, calcineurin.⁵⁵ In wild-type macrophages, upon internalization of mycobacteria, this phosphatase becomes activated, thereby blocking

phagosome-lysosome fusion by an as yet unknown mechanism and allowing survival of mycobacteria. In the absence of coronin 1, calcineurin activation does not occur, resulting in phagosome-lysosome fusion and intracellular killing of the internalized mycobacteria. Strikingly, genetic depletion of coronin 1 can be phenocopied by addition of the calcineurin inhibitors, cyclosporin A and FK506 (Figure 3). Thus, it appears that coronin 1 has evolved to activate Ca^{2+} -dependent signaling reactions in macrophages, thereby promoting the survival of pathogenic mycobacteria.⁵⁵

These results suggest that blocking the coronin 1 signaling pathway may allow intracellular killing of *M. tuberculosis*. Preliminary results indeed suggest that in vivo administration of calcineurin inhibitors allows rerouting of *M. tuberculosis* inside macrophages.⁵⁵ Because this newly discovered pathway is unlikely to be related to any of the mechanisms that are currently targeted by available tuberculosis drugs, we anticipate that blockers of the coronin 1 pathway may be useful for treatment of drug-resistant tuberculosis.

Therapeutic potential of agents blocking protein kinase G and coronin 1

Preliminary data have shown that blocking either the protein kinase G or the coronin 1 pathway may be highly specific for inhibiting growth of *M. tuberculosis* inside macrophages without affecting host functioning. For protein kinase G inhibition, a potent inhibitor of protein kinase G⁵³ was not active against a panel of 28 human kinases that were selected to represent the entire human kinome.⁵² In addition, when macrophages were exposed to high concentrations of these inhibitors, all measurable cellular functions appeared normal.

Figure 3 Mechanism of action of coronin 1 in intracellular survival of pathogenic mycobacteria.

With regard to coronin 1 inhibition, the clearest indication that coronin 1 can be blocked without obvious adverse effects is the phenotype of coronin 1-deficient mice. These mice develop normally, are healthy, and show no obvious phenotype.⁵⁵ In addition, the inhibitory protocol that we have developed to block the coronin 1-signaling pathway is based on use of cyclosporin A and/or FK506, both of which are drugs approved for use in humans.^{68,69}

Conclusion

It is reassuring that after many years, a tuberculosis drug pipeline is now developing. Moreover, several novel pathways essential for *M. tuberculosis* survival are currently being explored, including the protein kinase G and coronin 1 pathways, which have potential to serve as novel drug targets for treatment of both drug-sensitive and drug-resistant tuberculosis. Whilst pressing on with the development of drugs and optimized combination and usage of existing drugs at the “outcome” end of the pipeline, concerted effort is needed to expand further the portfolio of novel drug targets and to identify novel leads.

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Disclosure

The authors report no conflicts of interest in this work.

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