Towards new tuberculosis drugs

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Abstract
The need for better drugs to treat tuberculosis has never been greater. Despite insufficient funding for discovery research, intensive efforts have been made to find and develop new lead compounds capable of reducing the duration of the present treatment known as DOTS (directly observed therapy short course), from 6 to under 4 months. This mini-review describes the progress achieved during the last 5 years and highlights some of the successes without neglecting the problems.

Introduction
It is simply impossible to overstate the gravity of the current, global situation of TB (tuberculosis) as a public health menace. While the disease has plagued human societies for many centuries, an effective treatment for TB has only been available for 40 years. The WHO (World Health Organization) and the International Union Against Tuberculosis and Lung Disease recommend that DOTS (directly observed therapy short course) be implemented wherever possible. DOTS is a fully supervised treatment that lasts at least 6 months and comprises an initial intensive phase followed by the continuation phase. During the 2 month intensive phase, to prevent the emergence of resistant mutants patients receive three or four drugs (rifampicin, isoniazid, pyrazinamide and in many cases ethambutol), whereas, during the continuation phase, rifampicin and isoniazid are administered for a further 4 months to ensure sterilization.

While the exact number of cases of TB can never be known, the latest WHO survey estimates that close to 2 million deaths occur every year, that there are approx. 8 million new cases annually and that every third individual on the planet has been exposed to or infected with Mycobacterium tuberculosis [1]. The latter, latently infected population is at risk of presenting with the disease later in life as their cell-mediated immunity wanes due to malnutrition, aging or immunosuppression. The emergence of HIV and the resultant AIDS pandemic underlined the importance of reactivation disease and its potentially catastrophic outcome. Over 50% of deaths among the HIV-infected result from co-infection with Mycobacterium tuberculosis with the two pathogens triggering each other’s replication, thereby accelerating the collapse of the immune system. This raises questions about the logic of intensively pursuing anti-HIV measures while neglecting or inadequately funding TB research. It is ironic that dedicated agencies exist to tackle AIDS-related issues, while control of TB, for which breathing is the sole risk factor, lacks powerful advocates at the governmental and international levels. Unless there is a 10-fold increase in spending on TB research much of the success stemming from improved AIDS therapies may prove to have been ephemeral.

The 1990s saw the first cases of MDR-TB (multidrug-resistant TB), particularly among the homeless, drug abusers and the HIV-infected. MDR-TB, which is due to strains of Mycobacterium tuberculosis resistant to at least rifampicin and isoniazid, is increasingly common in parts of Asia, Russia and other states of the ex-Soviet Union [2]. However, a new, more deadly form has emerged among AIDS patients in South Africa that has been termed XDR (extensively drug-resistant) [3]. XDR strains are resistant to all components of DOTS and to many of the second-line drugs, including fluoroquinolones and the injectable aminoglycosides. Thus the spectre of untreatable TB looms greater than ever.

While there are many reasons for drug resistance, including prescription of inadequate regimens, insecure drug supply and ineffective drugs, the lengthy treatment duration is one of the major contributors; following their initial, rapid health improvement, some TB patients prematurely stop their therapy, thereby favouring the emergence of drug-resistant strains. In 2001, the Global Alliance for TB Drug Development published guidelines for drug discovery and highlighted a number of desirable properties for new drugs [4]. These should have increased potency, so that the duration of treatment could be reduced to 4 months at most; display activity against MDR-TB; kill the latent tubercle bacillus; be compatible with existing TB drugs, notably rifampicin; and act on new targets. In addition, it is desirable that a new TB drug shows no drug–drug interaction or antagonism with antiretroviral agents. Clearly, this is a challenging list of requirements that will be compounded by potential toxicity issues resulting from the long treatment duration.

Approaches to TB drug discovery
A variety of different approaches exist to discover new anti-infective agents. During the last decade, most emphasis has been placed on target-based methods, in

Key words: directly observed therapy short course (DOTS), rifampicin, PA-824, R207910, serine/threonine kinase, SQ109.

Abbreviations used: ADME, absorption, distribution, metabolism and elimination; DOTS, directly observed therapy short course; TB, tuberculosis; MDR-TB, multidrug resistant TB; WHO, World Health Organization; XDR, extensively drug-resistant.

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which an essential protein, usually an enzyme, is used in high-throughput screens of chemical libraries. Target identification has been driven by genomics [5] with data about gene essentiality arising from saturation transposon mutagenesis and conditional gene knockouts [6,7]. While successful in other therapeutic areas, target-based screens have been disappointing in antimicrobial agent discovery [8], usually because inhibitors that are active in vitro seldom cross the cell wall of the pathogen. Consequently, there is now growing interest in whole cell screens to uncover active compounds, although for TB this approach faces logistical constraints because of biosafety issues. Some investigators are trying to improve existing TB drugs as exemplified by work on derivatives of isoxyl, thiolactomycin and ethambutol. Other workers are investigating broad-spectrum antimicrobials, with notable recent examples being the fluoroquinolones and linezolid, or extending existing drug classes, as is the case with nitroimidazole derivatives.

Potential new TB drugs
This topic has been reviewed recently by several authors and their articles should be consulted for more details than can be given here [9,10]. Among the drug candidates currently being tested in clinical trials, or appraised for inclusion in enhanced regimens, are the latest generation of fluoroquinolones, the arabinogalactan inhibitor, SQ109, the diarylquinoline, R207910, newer nitroimidazole derivatives, and a variety of other compounds that show promise but have been less extensively investigated.

Fluoroquinolones
When MDR-TB first appeared, fluoroquinolones were tested for potential antitubercular activity. Ciprofloxacin was found to be insufficiently active as mutants with missense mutations in DNA gyrase soon emerged [11]. Newer derivatives such as sparfloxacin proved more effective but had unacceptable side effects. Improved success was achieved in animal models of TB with the latest fluoroquinolones, moxifloxacin and gatifloxacin [12], and this has been replicated in small trials in humans [13]. However, as the latter drug has just been withdrawn due to induction of dysglycaemia [14], future clinical trials will probably be limited to moxifloxacin, which lacks this side effect. Moxifloxacin was found to be highly bactericidal in a murine TB model and has the potential to shorten treatment duration considerably [15]. When isoniazid was replaced by moxifloxacin, mice were cured in 3 months of twice-weekly rifampicin and moxifloxacin, whereas with isoniazid and rifampicin, mice were cured in 6 months of pyrazinamide, rifampicin and moxifloxacin [16].

SQ109
Ethambutol is perceived as the weakest component of DOTS and thus most in need of improvement. Attempts have been made to synthesize more potent derivatives using combinatorial chemistry and from a library of over 60 000 diamine analogues, 26 compounds were identified that displayed activity equal to or greater than that of ethambutol [16]. Among these, three promising analogues (SQ37, SQ59 and SQ109) were selected as potential antitubercular drug candidates on the basis of their biopharmaceutical and pharmacokinetic properties, including stability in mammalian plasma and bioavailability [17]. SQ109 was retained for further development as a result of its favourable selectivity index, low MIC (minimum inhibitory concentration) (0.7–1.56 μM) and ability to inhibit 99% of the intracellular bacteria [18]. Detailed studies of the ADME (absorption, distribution, metabolism and elimination) of SQ109 have been performed [19] and the efficacy of the compound was recently assessed in association with isoniazid and rifampicin, where synergy was observed, and ethambutol or pyrazinamide, where no additive effects were seen [20]. Phase I trials are now in progress.

R207910
The discovery of the diarylquinoline, R207910, represents a major advance in TB drug discovery and merits extensive discussion [21]. Andries et al. [21] adopted an atypical approach in which not only did they eschew the target-based discovery paradigm in favour of a whole organism-based approach but they also used the fast grower Mycobacterium smegmatis instead of M. tuberculosis. Having identified their lead compound, these investigators then isolated resistant mutants of both mycobacteria and discovered the potential drug target by means of whole genome sequencing [21]. R207910 kills M. tuberculosis by inhibiting the c subunit of ATP synthase, an essential enzyme required for ATP production. The resistant mutants harbour missense mutations in the atpE gene encoding this subunit, and additional genetic, biochemical and drug-binding studies confirmed the authenticity of the c subunit as the target [22]. It is significant to note that the hydrophobic nature of AtpE, its universality and its belonging to a multisubunit complex would have precluded use of AtpE in a target-based screen, thereby underscoring the importance of multidisciplinary approaches to drug discovery.

In studies of experimental chemotherapy, synergistic activity of R207910 was observed with pyrazinamide in murine models of TB [23], and the drug also shortened the duration to culture negativity, particularly when associated with pyrazinamide, rifampicin and moxifloxacin [24]. Hopefully, R207910, which has a long half-life, will fulfill its promise in Phase II clinical trials and no adverse reactions will be encountered.

Nitroimidazole derivatives
It is generally accepted that adverse physiological conditions induce the latent state of M. tuberculosis with one such signal being hypoxia. Since nitroimidazole drugs only exhibit activity against microaerophilic or anaerobic bacteria it was logical to test these compounds for their ability to inhibit persisting tubercle bacilli. Metronidazole only displays weak antitubercular activity but more recent compounds such as PA-824 and
OPC67683 show strong bactericidal effects. PA-824 was isolated by library screening [25] and, like several other TB drugs, shown to be a prodrug requiring activation. Genetics and biochemistry were harnessed to identify the active form of the drug and its target but neither of these is truly clear at present. Activation involves an F420-dependent glucose-6-phosphate dehydrogenase and Rv3547 [26], a conserved hypothetical protein that might act as a nitroreductase, but the active species remains elusive. PA-824 has been tested in animal models in combination with other front-line drugs [27,28], where efficacy was sufficiently encouraging to justify Phase I trials. Like PA-824, the nitro-dihydroimidazo-oxazole derivative, OPC67683 [29], shows strong activity against M. tuberculosis in vitro and in mice, and is thought to inhibit mycolic acid production. Both these compounds show potential as antipersistence drugs, although their hydrophobic nature may lead to bioavailability problems in humans, so the results of ADME and toxicity tests are eagerly awaited.

Other approaches and concluding remarks

Some of the unusual biological features of M. tuberculosis open new vistas for drug discovery. For instance, somewhat unusually for a prokaryote, the tubercle bacillus produces no less than 20 cytochromes P450, some of which appear to play essential roles. In fungi, these enzymes are the target of azole drugs, which bind to the haem group leading to enzyme inactivation. Several investigators have therefore tested azoles for antitubercular activity with some success [30,31]. However, it has not yet been established whether the effects observed are due to inhibition of P450 function or to loss of unrelated activities. Another unusual characteristic of mycobacteria is their reliance on serine/threonine protein kinases as the main component of signal transduction pathways [32]. In the field of cancer, this class of enzymes has been intensively investigated and large kinase inhibitor libraries now exist, from which several clinically approved anticancer drugs such as Gleevec have been isolated [33]. Consequently, there is considerable activity around the mycobacterial serine/threonine protein kinases and recent work has shown some of these enzymes, notably PknB, to be essential for growth [34]. Furthermore, known kinase inhibitors not only block enzyme activity in vitro but also appear to kill the bacteria in broth cultures by inactivating these kinases.

There are major advantages in adopting an approved drug for use in TB treatment, such as the availability of extensive pharmacological and clinical information, and this should lead to quicker approval by the regulatory authorities. One such drug, tested under a compassionate-use programme for MDR-TB [35], is linezolide, an oxazolidinone antibiotic that targets the 50S ribosomal subunit [36]. Despite its antituberculosis effect in patients, linezolide induced some side effects such as peripheral neuropathy [37], possibly due to the long duration of therapy. There is hope, however, that the newer oxazolidinones will be better tolerated.

Some researchers have explored novel chemical space to detect pharmacophores for potential TB drugs. Among the antitubercular compounds synthesized de novo, which show high selectivity for mycobacteria, two families show some promise, the pyrazolobenzofurans [38,39] and the nitrofuranylamides [40–42].

The present minireview describes the current state of TB drug discovery and hopefully conveys the impression of much diverse activity and many avenues of investigation. It is clear, however, that attrition will take its toll and that, as in other drug development programmes, for every ten candidate drugs developed only one will ever reach the clinic. Furthermore, given the complexity and challenges of treating the disease, the discovery pipeline will need to be even more prolific and innovative than usual [43]. Bearing this in mind, it is essential to strengthen the effort to develop new TB drugs and this can only be done in a timely manner by increasing the funding available for discovery research.

We thank the European Commission for support (LHSP-CT-2005-018923).

References


Received 11 June 2007
doi:10.1042/BST035S1321