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Prospects for new antitubercular drugs

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The inexorable rise in cases of tuberculosis worldwide, fuelled by the HIV epidemic, highlights the need for new drugs and particularly those that can shorten the duration of treatment. Clinical trials of existing broad-spectrum agents such as the fluoroquinolone moxifloxacin are proceeding, on the basis of efficacy in models of infection and preliminary clinical data. These may provide a stopgap, but the real breakthrough will come when novel agents with potent sterilising activity are discovered. Few such novel pre-clinical drug candidates exist and therefore considerable effort is being exerted to employ new tools to identify drug targets essential for survival of *Mycobacterium tuberculosis*.

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Current Opinion in Microbiology 2004, 7:460–465

This review comes from a themed issue on Antimicrobials Edited by David Payne and Alexander Tomasz

Available online 9th September 2004

1369-5274/\$ – see front matter

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DOI 10.1016/j.mib.2004.08.011

Abbreviations

DOTS	directly observed therapy, short-course
EBA	early bactericidal activity
GATB	Global Alliance for TB Drug Development
LPP	large porous particles
MDR-TB	multidrug-resistant tuberculosis
MIC	minimum inhibitory concentration
PLG	poly(DL-lactide-co-glycolide)
TB	tuberculosis
TraSH	transposon site hybridization
WHO	World Health Organisation

Introduction

According to a recent report compiled by the World Health Organization (WHO), the total number of new cases of tuberculosis (TB) worldwide in 2002 had risen to approximately 9 million [1]. This is despite the undoubted success of widespread implementation of the 'DOTS' (directly observed therapy, short-course) strategy, now covering 180 countries and accessible by over 70% of the world's population. A key driver of the increase is synergy with the HIV epidemic, which is having a devastating impact in some parts of the world such as the WHO African Region, where 31% of new TB

cases were attributable to HIV co-infection [2]. Furthermore, the emergence of strains of *Mycobacterium tuberculosis* (MDR-TB), resistant to all the first-line drugs is causing serious concern in some countries [3].

No new classes of drugs for TB have been developed in the past 30 years, reflecting the inherent difficulties in discovery and clinical testing of new agents and the lack of pharmaceutical industry research in the area [4]. The Global Alliance for TB Drug Development (GATB; www.tballiance.org) was established to address this need. Its top priority is the development of a new agent that will shorten the duration of chemotherapy from the current 6–8 months to two months or less, although new drugs with activity against MDR-TB and latent TB are also needed [5]. There has also been considerable investment by both the private and public sector in the development of new agents for the treatment of TB but fundamental uncertainties in many aspects of the biology of the organism have substantially hampered the ability to identify critical targets whose inhibition would correlate with sterilising activity (Table 1). Sterilizing activity refers to the ability of a drug (such as pyrazinamide or rifampicin) to kill those organisms, known as 'persisters', that survive treatment with agents targeting essential processes in dividing bacteria. It is only by discovering new agents with improved sterilising activity that a shorter treatment regimen can be developed.

In this review, we discuss the drug candidates that are in clinical development and the efforts being made in pre-clinical research to exploit alternative delivery systems and to identify new drug targets.

Clinical studies

There have been no recent reports on the outcome of clinical studies with truly novel antitubercular agents, reflecting the previous lack of investment in drug discovery efforts. However, derivatives of known drugs or drugs developed originally for other antibacterial indications have been tested in TB patients.

Rifamycins

The most prominent of the new rifamycins is rifapentine. Its long serum half-life may permit establishment of an intermittent regimen, thus reducing the total number of dosages to be taken under DOTS supervision. However, it does not benefit from activity against rifampicin-resistant strains and is therefore unlikely to provide a breakthrough in therapy. In a pivotal phase III clinical study, rifapentine and isoniazid once per week was compared with rifampicin and isoniazid twice a week in patients

Table 1

Clinical trial endpoints	?	Microbiological endpoints
• Time to sputum conversion		• MIC
• Relapse rate after treatment		• Bactericidal/static activity
• Drug resistance		• Activity in animal models

One of the major impediments to developing new drugs for TB is the lack of understanding of how to predict the effect of what can be measured in microbiological studies *in vitro* and in animal disease models and what can be observed clinically in a human trial. 'Sterilising activity', a commonly referred to term in clinical trials, is not simply related to bactericidal activity as cidal activity is commonly measured against growing organisms while TB patients usually have a complex mixture of growing and non-growing organisms in a chronic infection.

who had completed two months of standard chemotherapy, that is during the continuation phase of treatment. The rifampentine regimen was found to be less effective in that a higher rate of drug-susceptible relapse was detected in HIV-negative patients, which correlated with the extent of cavitation [6]. In a follow-up pharmacokinetic study, low plasma levels of isoniazid were found to be associated with treatment failure or relapse, suggesting that an alternative companion drug may need to be identified for the intermittent rifampentine regimen to be fully effective [7].

Another rifamycin with a long half-life, rifalazil (previously known as KRM-1648), was investigated in a Phase II study. Patients were treated with rifalazil (10 mg or 40 mg) plus isoniazid for two weeks and compared with groups treated with isoniazid alone or isoniazid plus rifampicin. Comparable reductions in sputum bacillary load were found, and there were few drug-related adverse events [8].

Quinolones

A great deal of interest has been generated in the quinolone antibiotics following the publication of recent clinical and pre-clinical data confirming their potential for use in treatment of TB [9]. Data from a study of ofloxacin-containing regimens suggests that this drug (or a more potent quinolone) may be used to shorten the duration of chemotherapy. Patients treated with daily isoniazid, rifampicin, pyrazinamide and ofloxacin for three months, followed by one or two months of twice-weekly isoniazid and rifampicin had cure rates of 92–98% and relatively low relapse rates (2–4%) [10]. New generation quinolones possess significantly greater *in vitro* activity against *M. tuberculosis* than ofloxacin and therefore offer the greatest potential benefit to patients. Gatifloxacin and moxifloxacin are particularly active, having MIC₉₀ (minimum inhibitory concentration) values of 0.031 µg/mL and 0.125 µg/mL, respectively, compared with 1 µg/mL for levofloxacin [11]. The sterilising activity of various quinolones was investigated in a controversial *in vitro* model of persistence. Gatifloxacin and

moxifloxacin were found to kill rifampicin-tolerant bacteria much more effectively than levofloxacin or ofloxacin suggesting they may possess greater sterilising activity in the clinic [12].

Moxifloxacin has been examined in several *in vivo* models of *M. tuberculosis* infection aimed at guiding clinical development of the drug and at determining its possible role in TB therapy. Its long half-life suggested it might be a suitable companion for rifampentine, which was confirmed in experiments showing that addition of moxifloxacin to a rifampentine-containing regimen improved the efficacy and also revealing the potent sterilising effect of the drug [13]. Dose-dependent bactericidal activity in the mouse was found when moxifloxacin was given daily at up to 400 mg/kg, but not when given once-weekly [14]. Mice infected with an MDR-TB strain were successfully treated with moxifloxacin when combined with ethionamide [15]. In development of fluoroquinolone-containing 'third-line' regimens for treating MDR-TB, moxifloxacin was found to be a better agent to use than ofloxacin or levofloxacin, with sterilisation being achieved in nine months [16]. Using a murine model that mimics treatment in humans, it was found that addition of moxifloxacin to the standard isoniazid, rifampicin and pyrazinamide regimen had only a modest effect on bactericidal activity and did not result in an improved rate of tissue sterilisation. However, when moxifloxacin was substituted for isoniazid in the standard regimen, time to sterilisation was reduced by two months [17]. It is not clear whether this dramatic effect is due to synergy between moxifloxacin, rifampicin and pyrazinamide or relief from antagonism between isoniazid and rifampicin. It is important to emphasize that the pharmacokinetic antagonism between isoniazid and rifampicin that has been well documented in mice does not appear to be true in humans so truncation of therapy trials should be approached with caution [18,19].

Initial studies of moxifloxacin in TB patients have yielded encouraging results. The drug appeared to be well-tolerated over six months of therapy when given with rifampicin and isoniazid to a small group of patients with unusual TB manifestations who were not eligible for standard therapy [20]. The conventional route to developing new TB drugs often includes a Phase II study that measures early bactericidal activity (EBA), conducted by giving monotherapy over a period of up to five days at the start of treatment and measuring the reduction in colony forming units in sputum. Such studies are controversial, being most appropriate for those drugs that have potent bactericidal activity and less useful for predicting the sterilising activity of a new drug [19,21]. In Tanzanian patients receiving 400 mg moxifloxacin daily for five days, the measured EBA was found to be less than that of isoniazid, but greater than rifampicin [22]. The converse was found in a study conducted in Germany [23].

It remains to be seen whether resistance will limit the use of fluoroquinolones. High level phenotypic resistance in *M. tuberculosis* clinical isolates from patients in Hong Kong who had not received prior drug treatment was found predominantly to be associated with point mutations in DNA gyrase [24]. In a recent study in South Korea, 26% of patients with primary treatment failures showed resistance to ofloxacin suggesting that widespread use of fluoroquinolones for other infections may be contributing to the rapid loss of this class of molecule to the TB armory [25].

Pre-clinical candidates and alternative delivery systems

There are very few TB drug candidates at the pre-clinical testing stage, and little information is in the public domain indicating their progress. The most advanced is PA-824 [26], now being developed by GATB. This drug can now be synthesized in the large quantities required for animal and clinical trials, and in extensive animal testing no genetic damage nor toxic effects on normal metabolic or hormonal systems were identified [27]. This class of compound has potential to affect TB chemotherapy dramatically because of its demonstrated activity against anaerobic organisms [28].

From a library of over 63 000 diamines based on ethambutol, a compound was selected with improved *in vitro* activity against *M. tuberculosis* (MIC 0.2 μ M compared with 7 μ M for ethambutol) [29^{*}]. In a murine model of *M. tuberculosis* infection, efficacy of this compound, now known as SQ109, was observed at 1 mg/kg comparable with 100 mg/kg ethambutol, and it is reported by Sequella Inc to be in pre-clinical development [30].

Alternative systems have been investigated to determine whether the currently-available drugs can be delivered more efficiently and effectively. Subcutaneous or oral delivery of sustained release formulations might permit intermittent chemotherapy thus improving compliance. Delivery by inhalation may allow drugs that are poorly bioavailable or have systemic side-effects to be used, or else deposition at the site of infection may reduce the bacterial burden more rapidly. Investigations are at a relatively early stage and are currently focusing on demonstrating activity in *in vivo* models of infection. Further experimentation will be required to determine whether additional toxicity arises following delivery by a different route and pharmaceutical development will need to be undertaken to generate stable large-scale preparations before clinical development can be considered. Cost and convenience will be major factors in determining whether alternative delivery systems, if successful, will actually be used.

Entrapment of TB drugs in poly(DL-lactide-co-glycolide) (PLG) microspheres has been investigated extensively.

A single subcutaneous administration of isoniazid and rifampicin PLG microspheres to mice was shown to provide a sustained release of the drugs over seven weeks and reduction in colony forming units comparable with daily oral drugs [31]. Orally-available formulations of PLG microspheres have been developed that contain isoniazid, rifampicin and pyrazinamide. Following a single administration, drug levels peaked after three days and were sustained above the MIC for nine days [32^{*}], suggesting that weekly dosing is feasible. Five weekly doses of drug-loaded PLG microspheres was found to be as effective as 35 days treatment with free drugs [31^{*}]. An even more striking result was obtained when *M. tuberculosis*-infected mice were treated every ten days with PLG microspheres for 46 days, when no bacilli were detected [33]. Alginate-encapsulated oral preparations have also been investigated [34,35].

Drug-loaded PLG microspheres have also been delivered to the lung by insufflation and nebulisation methods. Delivery of rifampicin this way to guinea pigs before infection with *M. tuberculosis* reduced the subsequent burden of infection [36]. Isoniazid and rifampicin PLG microspheres were found to be rapidly phagocytosed by rat alveolar macrophages achieving intracellular drug concentrations higher than orally delivered drugs [37]. Large porous particles (LPP) can be used to deliver large quantities of drug. When a para-aminosalicylic acid-LPP formulation was delivered by insufflation to rats, therapeutic drug levels were obtained in the lung at lower total body dose than by oral exposure [38].

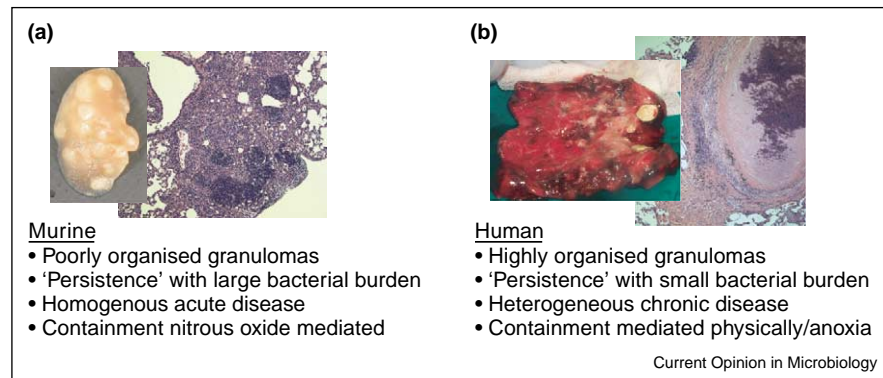
Drug discovery efforts

Much of the research effort in TB drug development is addressing the early stages of the pipeline, including basic research aimed at identifying and validating drug targets and screening for lead compounds, although only a few leads are being optimised to generate drug candidates [39]. Several strategies are being pursued in order to identify new leads. These include making derivatives of existing drugs and screening for activity against *in vitro* cultured whole cells, isolated essential targets, *in vitro* models mimicking persistence, and targets required for survival only in the human host [40]. Advances in basic research in TB of particular relevance to drug discovery are described below.

Genomics

Analysis of the *M. tuberculosis* H37Rv and CDC1551 genome sequences [41,42^{*},43] revealed a great deal about the biology of the pathogen. Subsequent comparison with genomes of *Mycobacterium leprae* [44] and other mycobacteria has suggested which members of certain multigene families may be important [45] and a 'core' set of mycobacterial genes that could include highly selective drug targets [45,46].

Figure 1



An imperfect animal model. Murine TB does not reproduce many of the critical features of human TB. **(a)** A photo of an infected mouse lung and a hematoxylin and eosin stained slide showing the homogeneous, loosely organized nature of the disease in mice. **(b)** A photo of a human lung, removed during lung resection surgery and a hematoxylin and eosin stain of a single granuloma structure that is highly organized and structured, with discrete bacterial populations at different physical locations. Drug development for human TB suffers seriously from over-reliance on the mouse model of experimental chemotherapy.

New tools for drug target identification and validation

Specific and comprehensive approaches have been applied to identify essential *M. tuberculosis* genes, in particular to reveal targets that may be important for survival in the host [47]. The most successful technique is transposon site hybridisation ('TraSH'), which generates pools of mutant bacteria by saturating transposon mutagenesis then uses a DNA microarray-based technique to locate the insertion points on the chromosome. Genes that are not mutated are therefore predicted to be essential under the particular growth conditions. TraSH was initially used to identify genes required by *Mycobacterium bovis* BCG to grow on minimal and not on rich media [48], and has since been applied to the study of genes required for optimal *in vitro* growth of *M. tuberculosis* [49], and for survival during infection [50]. In the latter case, 194 genes were required for *in vivo* growth, many of which are unique to mycobacteria [50]. The lack of a useful regulatable promoter for use in *M. tuberculosis* continues to hamper attempts to generate reliable target validation evidence in models of infection.

DNA microarrays have been employed to monitor simultaneously the expression of all *M. tuberculosis* genes under a variety of different growth conditions and stresses. In one study, a 48-gene regulon was discovered, which appears to be expressed when *M. tuberculosis* enters dormancy [51] a finding which may point the way to future studies aimed at defining targets essential for survival in the latent state.

Drug targets and lead compounds

Biogenesis of the mycobacterial cell wall has classically been regarded as a rich source of selective TB targets [52,53] and attempts have been made to find inhibitors of

polysaccharide [54] and fatty acid [55] biosynthesis. Many other aspects of the biology of the organism have also been considered, including genes thought to be involved in persistence, and various regulatory enzymes [56,57]. These genes have been mutated and the resulting strains used to infect animals to determine whether there is an effect on the course of disease. Unfortunately, most studies to identify critical targets have been performed using murine models of chronic disease, which only poorly mimics human tuberculosis. The lack of a well-accepted model for human chronic disease is a second major stumbling block for improving therapy for TB (see Figure 1). Little if any comprehensive screening has been carried out to identify inhibitors of isolated targets and there are no literature reports of leads derived from such efforts. Instead, the majority of the lead compounds described in the literature in recent years were identified on the basis of their activity against whole cells. It remains to be seen whether these will yield drug candidates in the absence of a knowledge of the specific target.

Conclusions

Few new agents for treating TB are in development today, and none has been designed specifically to shorten the treatment regimen and provide the breakthrough in therapy that is sorely needed if the TB epidemic is to be brought under control. Of drugs developed for other indications, moxifloxacin shows greatest promise, although emerging resistance to fluoroquinolones may limit its use. Alternative delivery systems may provide ways to maximise the benefit of today's drugs but it is unclear whether they can be produced at an affordable cost. Our improved knowledge of *M. tuberculosis* biology is identifying potential new drug targets. Further investment in developing fundamental genetic systems and more accurate models

of human disease would significantly facilitate TB drug discovery efforts in the long term, in particular enabling robust validation of novel targets. It will be many years before the drug targets now identified can be fully exploited and novel drug candidates generated.

Update

Recent work has compared the transcriptional profiles of *M. tuberculosis* to drugs and inhibitors of all major metabolic processes including most of the known drugs with antitubercular activity. This work allows for the possibility of *de novo* mechanism of action determinations and has been used to reveal a large number of co-ordinately regulated gene groups that provide essential clues for the rational selection of targets and a detailed understanding of the physiology of non-replicating bacteria [58].

Acknowledgements

We thank Dr. Laura Via (TBRS, NIAID) for the photographs of murine and human TB shown in Figure 1.

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