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Pharmacologically active metabolites, combination screening and target identification-driven drug repositioning in antituberculosis drug discovery

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ABSTRACT

There has been renewed interest in alternative strategies to address bottlenecks in antibiotic development. These include the repurposing of approved drugs for use as novel anti-infective agents, or their exploitation as leads in drug repositioning. Such approaches are especially attractive for tuberculosis (TB), a disease which remains a leading cause of morbidity and mortality globally and, increasingly, is associated with the emergence of drug-resistance. In this review article, we introduce a refinement of traditional drug repositioning and repurposing strategies involving the development of drugs that are based on the active metabolite(s) of parental compounds with demonstrated efficacy. In addition, we describe an approach to repositioning the natural product antibiotic, fusidic acid, for use against *Mycobacterium tuberculosis*. Finally, we consider the potential to exploit the chemical matter arising from these activities in combination screens and permeation assays which are designed to confirm mechanism of action (MoA), elucidate potential synergies in polypharmacy, and to develop rules for drug permeability in an organism that poses a special challenge to new drug development.

1. Introduction

Tuberculosis (TB) is a global problem: there were around 8.6 million new cases in 2012, with the disease claiming approximately 1.3 million lives.[1] Moreover, it is estimated that one-third of the world's population (approximately 2 billion people) is infected with the causative agent, *Mycobacterium tuberculosis*.[2] There are a number of endemic regions which carry the greatest burden of disease: of these, South Africa ranks third behind only the world's most populous countries, India and China. By far the largest proportion (approximately 80%) of HIV-positive incident TB cases are in Africa, with South Africa, which has only 0.7% of the world's population, contributing a huge portion of these at ~25% of all HIV-TB co-infections.[3–5] In addition to HIV, multiple factors continue to undermine TB control measures in endemic areas, including the increasing emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) *M. tuberculosis* strains that are resistant to the major anti-tubercular agents, the variable availability and inconsistent quality of the frontline anti-TB drugs, the prevalence of other chronic diseases that can increase TB morbidity, and numerous sociological confounders.[6] Although a vaccine exists, the widely administered BCG is effective only against the most severe forms of pediatric TB disease and, critically, offers no protection against adult pulmonary TB.[7] Instead, the major thrust of global control efforts is devoted to chemotherapeutic intervention in active disease, utilizing a combination therapy that extends over a minimum six-month treatment period comprising a two-month intensive phase with four drugs and a four-month continuation phase with two drugs.[8] For MDR-TB, the treatment duration is extended to a minimum of 9-12 months, and utilizes drug combinations that are less easily administered, less well tolerated, more expensive, and carry a much higher risk of failure. XDR-TB and, more recently, totally drug resistant (TDR)-TB pose an even greater therapeutic challenge, and can be almost untreatable.[9]

For a disease that ranks behind HIV only in mortality owing to a single infectious agent, it is sobering that the majority of the drugs used to treat TB were developed 50–60 years ago.[6] Recent years have witnessed a massive shift, however, and new drug discovery

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represents a major activity (and funder) of TB research laboratories worldwide. Against this background, we have committed to developing a TB drug discovery programme at the University of Cape Town, which includes routine compound screening and structure activity relationship (SAR) testing of chemical matter from a variety of sources, with a particular focus on synthetic compounds generated as part of an active medicinal chemistry programme.[4] Increasingly, these activities include novel approaches to maximize the potential to identify and/or develop compounds that are active against the causative agent, *M. tuberculosis*. In this short review, we discuss two strategies employed in our laboratory involving exploration of chlorpromazine metabolites as part of a drug repurposing strategy, and the investigation of the natural product antibiotic, fusidic acid, as a vehicle for drug repositioning.

2. New TB drug discovery

The urgent need for new anti-TB chemotherapeutics has prompted unprecedented investment in all stages of drug discovery and development. However, rates of attrition are extremely high, especially in the clinical phases, necessitating a constant supply of novel chemical matter into the development pipeline. In general, drug discovery for infectious diseases is heavily reliant on the synthesis of novel chemical entities, or their isolation and development from natural products. More recently, however, there has been renewed interest in the possibility of exploiting alternative strategies to address the well-documented bottlenecks in antibiotic development.[10] These include the potential to repurpose approved drugs for use as novel anti-infective agents,[11–14] or to use them as leads in drug repositioning strategies. Such approaches are especially attractive for TB, a disease which, despite the availability of effective frontline chemotherapy, remains a leading cause of morbidity and mortality in many countries in the developing world and, increasingly, is associated with the emergence of drug-resistant strains of the causative agent, *M. tuberculosis*.[15] In this review article, we introduce an additional strategy, pursued in our laboratory, involving the development of drugs that are based on the active metabolite(s) of parental compounds with demonstrated efficacy. We also describe one approach to drug repositioning for *M. tuberculosis*, with special reference to fusidic acid, a natural product antibiotic used in Europe and Australia in the treatment of skin infections caused by Gram-positive bacteria.[16] In addition, we briefly consider the potential to exploit the chemical matter arising from these activities in downstream applications, such as combination screens and permeation assays, which are designed to confirm mechanism of action (MoA), elucidate potential synergies in polypharmacy, and to develop rules for drug permeability in an organism that poses a special challenge to new drug development.[6,17–19]

3. New approaches to TB drug discovery

3.1. Exploring active metabolites as drugs

Metabolism is the process that converts compounds into derivative chemicals that can be more readily eliminated from the body. Where the parental compound is a drug, the products of metabolism can include metabolites which are active against the same pharmacological target as the administered molecule.[20] The formation of pharmacologically active metabolites is usually mediated by two mechanisms: the most common of these, phase I metabolism, depends on cytochrome P450 enzymes and includes oxidation, reduction, and hydrolysis, whereas phase II metabolism occurs via conjugation reactions.[21] By definition, metabolism of the active metabolite(s) itself will lead to the formation of fewer total metabolites than are obtained from the parent compound. The use of an active metabolite as a drug is attractive in that it may reduce off-target toxicity: by definition, reducing the number of metabolites that can be generated from the applied drug should limit the possibility for unwanted effects which can result from the inhibition of essential host functions.[22]

The chemical and structural characterization of metabolites derived from novel lead compounds is generally not completed until late in the drug development process. It is at this point that the relative contribution of active metabolites to the observed therapeutic effect is evaluated. In some cases, however, a full assessment of the pharmacological significance of metabolites is not initiated until after drugs have reached the market.[21] Moreover, while major technological advances have enabled early determination of biotransformation profiles, it nevertheless remains uncommon for drug candidates to be screened for the presence of active metabolites during lead optimization. As a result, biological transformation as a method of drug design has not been widely exploited,[21] notwithstanding the fact that those pharmacologically active metabolites which have been successfully developed as drugs often possess more desirable physicochemical, pharmacodynamic, and pharmacokinetic properties compared to the respective parental compounds (Table 1).[21,23–25]

It is notable, in particular, that there are no reports of antimycobacterial agents developed from active metabolites. This suggests an area of opportunity for medicinal chemists in the context of TB drug discovery. In section 4 (below), we describe our exploration of chlorpromazine as repurposed anti-TB agent.

3.2. Combination screening

Repurposing or repositioning of existing drugs[14,26] represents an attractive approach to overcome the limited ability of recent screening activities to identify viable hit compounds against *M. tuberculosis*.[19] Although the terms 'repurposing' and 'repositioning' have been used interchangeably in the literature, drug repurposing refers specifically to cases in which an existing drug, approved by a regulatory agency in one disease area, is found to have activity against another disease. In contrast, drug repositioning describes a situation where a drug that is active in one disease is used as a template for the synthesis of derivatives active in another disease.

Examples from unrelated fields such as cancer drug discovery suggest the potential for simultaneously targeting more than one pathway or pathway component[27] as part of fractional combination therapies designed to achieve synergies in drug action. Drug synergy occurs when the interaction between two or more compounds enhances the individual activities of each. There are a number of examples of drugs that have been developed in combinations designed to increase efficacy while limiting the emergence of drug resistance. These include artemisinin-based combination therapy (ACT) for the treatment of malaria, and antiretroviral drugs (ART) for the management of HIV/AIDS.[14,28,29] Given that combination therapy is standard for TB (Fig. 1),[3] it seems sensible to include combination screening in the early stages of drug development in order to identify potential partners for novel anti-TB drug regimens.

Table 1
MIC_{99} of CPZ and its metabolites[47]

Drugs/compound	MIC_{99} (μM) in *M. smegmatis*
CPZ	117.26
CPZ sulfoxide	>1990.89
7-Hydroxycpz	124.44
CPZ-*N*-oxide	995.43
nor-CPZ	136.70
nor-CPZ sulfoxide	>2077.89
CPZ-*N*-*S*-dioxide	>1900.10