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A SHORT REVIEWON DRUG RESISTANCE AND COMPLICATIONSIN THERAPYOF TUBERCULOSIS

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Abstract

Chemotherapy is the most potent therapy for treatment of tuberculosis. In the chemotherapy of tuberculosis, resistance associated with treatment failures emerged and has become a common occurrence all around world. The multi-drug therapy is used to prevent the emergence of drug resistance mutants during the long duration of treatment. Resistance can be defined as single-drug, multi-drugs, depending on the number of drugs and/or which drugs are involved. The drug resistance tuberculosis more prevalent, lack the resources to implement adequate measures to control even the susceptible types of the disease. A high prevalence of primary multidrug-resistant tuberculosis, previous treatment for TB predisposes to the selection of multi drug resistance organisms and non-compliance is a major factor in allowing the resistant organisms to survive.

Keywords:*Chemotherapy, tuberculosis, resistance, drugs.*

1. Introduction

Mycobacteriumtuberculosisinfects about 32% of the world's population. Every year, approximately 8 million of tubercular infected people develop active tuberculosis (TB) and almost 2 million of these will die from the disease [1]. Despite 40 years of anti-TB chemotherapy, TB remains one of the leading infectious diseases worldwide. Among the main obstacles to the global control of the disease are the human immune deficient virus (HIV) epidemic that has dramatically increased risk for developing active TB, the increasing emergence of multidrug resistance (MDR) TB and the revolt of persistent infections to treatment with conventional anti-TB drugs [2,3]. The situation is exacerbated by the increasing emergence of extensive drug resistance (XDR) TB [4]. The XDR-TB is characterized by resistance to at least the two first-line drugs like rifampicin (RIF) and isoniazid (INH). Current chemotherapy for TB largely relies on drugs that inhibit bacterial metabolism with a heavy emphasis on inhibitors of the cell wall synthesis [5]. According to their mode of action, first and second line anti-TB drugs can be grouped as cell wall inhibitors (Isoniazid, ethambutol, ethionammide, cycloserine), nucleic acid synthesis inhibitors (Rifampicin, quinolones), protein synthesis inhibitors (Streptomycin, kanamycin) and inhibitors of membrane energy metabolism (Pyrazinamide). Existing TB drugs are therefore only able to target actively growing bacteria through the inhibition of cell processes such as cell wall biogenesis and deoxyribonucleic acid (DNA) replication. This implies that current TB chemotherapy is characterized by an efficient bactericidal activity but an extremely weak sterilizing activity, defined as the ability to kill the slowly growing or slowly metabolising bacteria that persist after the growing INH and additionally to a fluoroquinolone and an injectable drug (kanamycin, amikacin or capreomycin) among the second-line drugs. The extensive resistance makes this form of TB particularly cumbersome to treat with available drugs. The current situation clearly demonstrates the need for a re-evaluation of approach to treating TB. Drug development for TB and other infected diseases has been at a practical standstill for decades, but increased awareness and advocacy in recent years have led to new initiatives in TB drug development [6-10].

2. Chemotherapy and management of TB:

Isoniazid (INH), rifampin (RIF), ethambutol (ETH) and pyrizinamide (PZA), administered as one combination tablet during the intensive phase (first two months) of TB treatment, form the direct observation therapy short course (DOTs) strategy recommended by the world health organization (WHO) for the treatment of TB. Streptomycin (STR) is added in the treatment regimen in cases previously treated for TB. Other drugs, such as amikacin and ciprofloxacin, may be added or substituted. The success of treatment is dependent on two factors, that is, the sensitivity of the organisms to drugs in use and the risk of severe toxic effects produced by these agents. Unlike most infections treated with antibiotics, the period of TB treatment is measured in months and years. Long-term compliance therefore is one of the important challenges in control of the disease [11]. The DOTs plus system has been suggested. The increasing occurrence organisms are resistant to isoniazid (INH) and rifampicin (RIF), these two drugs form the backbone of DOTS therapy. Rifampin (RIF) resistance occurs mostly in conjunction with INH resistance (90% of cases) and can be used as a substitute marker for multidrug resistance (MDR) and extensively drug-resistant (XDR) TB. According to the WHO, the principal reason for the spread of MDR-TBis ineffectual management of TB control programmes, particularly in developing countries. Suboptimal administration of drugs not only leaves the patient still sick and still infectious, but also favours the selection of resistant bacteria [12-15].Currently the WHO urges that TB programs worldwide adopt the practice of DOT. TB control programme are

1) to reduce mortality and morbidity attributable to TB,
2) to prevent the development of drug resistance, and
3) to ensure accurate measurement and evaluation of programme performance.

Short-term objectives are

1) to achieve smear conversion rates of at least 85% among new smear positive cases and 80% among retreatment cases at the end of the intensive phase of treatment, and

2) to cure at least 85% of new smear positive cases with short course chemotherapy.

Prevention involves identification and subsequent treatment of sputum-positive patients, finding active cases of infection among their close contacts and the vaccination of all children with attenuated *M. bovis*BCG (bacillus Calmette-Guerin). All children under 5 years living in the household of the active index case receive prophylactic treatment with INH. Drugs for TB are administered simultaneously as a single tablet (INH, RIF, PZA and EMB) for 2 months (induction phase) and for the next four months (continuous phase) INH and RIF are administered as a combination tablet. Although therapy is usually given for months, the patient's sputum becomes noninfectious within a couple of weeks [16-18].
Protracted therapy is attributed to

1) the intracellular location of the organism,
2) caseous material, which blocks penetration by the drug,
3) the slow growth of the organism, and
4) metabolically inactive organisms within a lesion.

Anti-TB drugs may not kill metabolically inactive organisms, treatment may not eradicate the infection and reactivation of the disease may occur in the future [19,20].

3. Interaction of TB and HIV/AIDS:

The advent of the HIV/AIDS pandemic has fuelled the spread of TB worldwide. The rate of TB patients that were HIV positive reached more than 60% than HIV negative patient. Persons co-infected with HIV and TB has an increased risk of developing active TB. The result is an increase in TB cases among non-HIV infected persons due to a larger pool of source cases in the community [21]. A disturbing issue is that this increase in the incidence that threatens to overwhelm TB control programmes will inevitably be accompanied by a rise in drug resistance TB. The best approach to reduce the increasing TB case load attributable to HIV infection will be to complement the DOTS with DOT-plus strategies that will rapidly identify MDR strains and their susceptibility patterns. The association between TB and the HIV infection is threatening to overwhelm control programmes globally.

4. Development of new drugs for TB chemotherapy:

With approximately 9 million people developing active TB every year and 1.7 million deaths annually, TB is far from under control. HIV infection dramatically increases the risk of developing active tuberculosis and is driving the TB epidemic in Africa. HIV renders TB more difficult to diagnose (due to higher incidence of sputum negative disease), and treat (due to interactions and side-effects). The increasing spread of MDR-TB and the recalcitrant nature of persistent infections pose additional challenges to treatment with currently available anti-TB drugs. The situation is exacerbated by the increasing emergence of XDR-TB. Resistance to at least two main first-line drugs and additionally to three or more of the six classes of second-line drugs makes this form of TB virtually untreatable with available drugs. Although TB can be cured, current treatment is complex and long lasting, involving four drugs for 2 months and two drugs for at least another 4 months. Direct obseve therapy short course, as promoted by the WHO to improve compliance for the difficult and long-lasting regimen, is demanding for patients, labour intensive for health staff and is compromised in settings where health services are poorly accessible. MDR-TB is even more complex and expensive to treat, and in developing countries treatment is limited to a few projects with limited numbers of patients. After decades of standstill in TB drug development, the drug pipeline has begun to fill up during the last 5 years. The main criteria established to select drug candidates for further development are shortening of the current treatment, activity against MDR-TB and lack of interactions with antiretroviral drugs represent. Modern molecular and genetic tools have become available for *Mtb* (such as targeted mutagenesis, array-based analysis of mutant libraries, techniques for conditional gene silencing, and global gene expression profiling) and this has led to impressive improvements in the knowledge and understanding of the basic biology and physiology of *Mtb*. Despite these positive changes there are still problems that need to be tackled. A critical question today is whether they are sufficient to bring improved treatment to patients in the next few years. A first challenge concerns the sustainability of the current effort [22-26]. As promising compounds move into expensive clinical trials, such as the TB Alliance face a significant funding gap. Financial support will need to increase to ensure that the development of these promising new compounds is supported all the way to trials. Sufficient numbers of promising compounds in the TB for a broadly effective new treatment combination to be developed. Although, different attrition rates might apply the number of candidate compounds is still small compared to the drug pipelines for diseases of major concern. Furthermore, many of the compounds in the pipeline are either derivatives of existing compounds or they target the same cellular processes as drugs currently in

use. Whilst analogues and derivatives are far quicker to develop, they may be subject to cross-resistance, as has been the case with the new rifamycins and quinolones. Modern technologies and rational approaches to drug design (such as creation of genomic mutants for comprehensive target identification and validation, target-based drug discovery, or determination of three dimensional crystal structure of molecular targets) are still weakly implemented in the field of drug discovery for tuberculosis. Even the more promising candidate compounds currently in clinical development were identified serendipitously in screenings that were not designed originally for activity against *Mtb*. There is consensus among the TB scientific community that in order to obtain a real breakthrough in TB therapy and drastically shorten treatment there is an urgent need for rational approaches aimed at tackling the problem of mycobacterial persistence. The adaptations that allow *Mtb* to persist in the host despite a vigorous adaptive immune response likely contribute to the difficulty in curing TB with current chemotherapy [27-30]. Although drugs currently in the pipeline could significantly shorten treatment, it is likely to remain a matter of months rather than weeks or days.

As emphasized for TB Drug Development [31] a new TB treatment should offer at least one of the following three improvements over the existing regimens:

• shorten the total duration of treatment and/or significantly reduce the number of doses needed to be taken under DOTs,

• improve the treatment of MDR-TB, and

• provide a more effective treatment of latent TB infection Shortening of the current treatment, activity against MDR-TB, and lack of liver enzyme induction and inhibition (to avoid interactions with antiretrovirals) are the main criteria the TB Alliance is using to select drug candidate that should be pursued for further development. Finding a treatment for latent TB is currently not a strategic priority for the TB Alliance as it considers treatment of active TB as a more feasible achievement to be reached in a shortterm perspective.In order to shorten the development time for a new regimen, working on both identifying individual novel compounds and developing new drug combinations. TB Alliance is currently engaged in discussions, how they can test new compounds simultaneously rather than consecutively. Indeed, the conventional approach to drug development requires to substitute each drug in the current regimen singularly, only after each new drug has been approved. Considering that it takes on average 6 years for a new drug to be registered, the development of a completely new first line regimen could take approximately 24 years. TB Alliance's innovative proposal of testing new compounds simultaneously could drastically shorten the procedure, but ethical implications have to be taken in strong consideration in identifying a practical way to implement such clinical trial design [32]. Novel chemical entities and compounds originating from existing families ofdrugs, where innovative chemistry is used to optimise the compounds under random clinical trial conditions are often 3% or less, large numbers of patients are needed to demonstrate an improvement in relapse rate. This results in high drug development costs and long delays in introducing new medicines [33]. Less well validated procedures that require further studies and validation are the rate of sputum conversion, the measurement of the 85B (alpha) antigen of *Mtb* in sputum and the extended studies (beyond 2 days) of early bactericidal activity (EBA) [34]. However, regulatory agencies still require that drug's efficacy is demonstrated during phase III trials through a combination of traditional and surrogate markers for activity. Since the identification of biomarkers could significantly streamline and accelerate clinical development. A biomarker is a quantifiable biochemical characteristic (like metabolite, hormone or enzyme) that is measured and evaluated as a pharmacologic response to chemotherapy.

5. Targets and mode of action of current TB drugs:

Bacteria have been killed by bactericidal drugs. Sterilizing activity also describes the ability to eliminate latent or "dormant" bacteria that survive inside the host macrophages. This bias is hardly surprising as anti-TB drugs have traditionally been identified by their ability to suppress or kill replicating cultures of bacteria in vitro. The weak sterilizing property of available TB drugs is one of the major drawbacks for current TB chemotherapy. Although RIF and PZA are partially sterilizing drugs and play an important role in shortening the therapy from 12-18 months to 6 months, there are still populations of persisting bacteria that are not killed by RIF and PZA. Thus, although achieving a clinical cure, the current TB chemotherapy does not achieve a bacteriological cure since the therapy cannot completely eradicate all bacilli in the lesions. HIV has dramatically increased the risk of developing active TB and HIV co-infection makes TBs more difficult to diagnose (due to more complicated presentations) and treat (due to interactions and side-effects). The increasing emergence of MDR-TB and the recalcitrant nature of persistent infections pose additional challenges to treatment with conventional anti-TB drugs. Although TB can be cured with current drugs treatment is complex and long-lasting, involving four drugs for two months and two drugs for at least another 4 months. This makes compliance difficult. DOTS as promoted by the WHO to improve compliance for the difficult and long regimen can improve cure rates but is demanding for patients and labour intensive for health staff [35-38]. The efficacy of drugs against *Mtb* in vitro was not matched by their efficiency in vivo. The difference is striking; exponentially growing cultures of *Mtb* can be sterilized in a few days using frontline bactericidal drugs such as INH and RIF, yet the same drug combination requires months to achieve similar effects against bacteria living in host tissues. The most obvious explanation would be failure of drugs to achieve optimal levels within TB lesions, but there is evidence that drugs availability is not a limiting factor. It has been proposed that persistence of tubercle bacilli in the face of chemotherapy might be attributable to physiologic heterogeneity of bacteria in the tissues [39-42]. This idea was inspired and supported by the long-established observation that slow- and non-growing bacteria are phenotypically resistant or tolerant to killing by antimicrobials). The tubercle bacilli in lesions consist of at least four different populations:

1) Bacteria that are actively growing, killed primarily by INH
2) Bacteria that have spurts of metabolism, killed by RIF
3) Bacteria that are characterized by low metabolic activity and reside in acid pH environment, killed by PZA
4) Bacteria that are "dormant" or "persisters", not killed by any current TB drug.

During the initial phase of chemotherapy, which lasts for about 2 days, bacilli are killed exponentially at a rapid rate, followed by a further lengthy period of much slower exponential killing. It is assumed that those bacilli killed in the first 2 days are actively multiplying, while those in the succeeding period are persisters killed by the slower sterilizing activities of the drugs. As mentioned in the previous section, drugs in the current regimen differ in their relative bactericidal activities, with the activity of INH predominating during the initial phase, and in their subsequent sterilizing activity, with the activity of RIF and PZA predominating during the continuation phase [43]. In an in vitro model of drug action, a 30-day static culture has been extensively used for the last 60 years and has been taken to resemble the persister population in its response to drugs [44]. The drugs added to this static culture have the same slow sterilizing action that is responsible for the prolongation of therapy. Thus, evidence suggests that activity against the population of persistent bacilli ultimately determines the duration of therapy necessary for a given regimen to sterilize lesions and provide a stable cure of the host [45-47]. From this

there is evidently an urgent need to develop new and more effective TB drugs that are not only active against MDR-TB but also shorten the length of treatment targeting non-replicating persistent bacilli.A systematic characterization of the heterogeneity of TB lesions in patients aimed to get a clearer picture of the different microenvironments that bacteria have to adapt to in order to survive and persist in human hosts, is still largely missing. Similarly, the understanding of the critical mechanisms that underlie survival of *Mtb* during the extended periods of chemotherapy is still rather limited. These gaps in our knowledge of *Mtb* biology are making the identification and validation of potential targets that are relevant in vivo in the human host still a rather difficult task. The "biological uncertainties" about *Mtb* also represent one of the reasons why pharmaceutical companies consider anti-TB drug discovery and development as particularly risky ground and are therefore generally reluctant to embark on this kind of projects. In an effort to develop drugs for latent TB, is aiming to identify the molecular pathways essential for the bacteria to survive inside human tissues. The purpose is to select targets that are essential for viability of "persistent" bacteria that are relatively tolerant to conventional drugs. Another "scientific obstacle" that is currently under debate is the lack of adequate animal models truly representative of human latent TB. Although there is a consensus that mice, rabbits, guinea pigs and non-human primates infected with *Mtb* can model overlapping characteristics of human TB, some scientists strongly advocate for the use of non-human primates as the animal model of choice [48,49]. Currently, the most popular model is the murine model due to low cost, availability of genetically defined strains and comprehensive characterization of mouse immunology. While there are similarities in the immune control of TB in mice and humans, the progression of the disease is markedly different. The features of chronic infections in mice (high bacterial titre in the lungs and spleen, absence of necrotic lesions, precursors of cavities) do not reflect the situation of advanced TB in humans [50,51]. However, the choice of the "right" animal model requires a previous systematic characterization of TB lesions in the human hosts. As far as this characterization will be largely missing it might be premature and is not really evidence-based to advocate for one or another animal model. Lack of sustained funding for early stage drug discovery projects is the other barrier in the process of anti-TB drug discovery. Examples are target validation projects or projects in the field of chemical genetics[52,53].

6. Pressing needs still remain:

Despite the positive changes occurred in the last years, there are still problems that need to be tackled and major roadblocks still exist that are hindering the implementation of rational drug design and a fast progress in anti-TB drug research and development. A first important question is if there are enough promising compounds for a comprehensive new TB treatment to be developed [54]. Although different attrition rates might apply, the number of candidate compounds is still small if compared to drug pipeline for diseases that principally affects wealthy countries. This is reflected by the limited number of biotech and pharmaceutical companies working on TB. The ambition of the TB Alliances is to register an improved, faster acting regimen and a regimen containing completely novel drugs. It is evident that many of the candidate drugs are either derivatives of existing compounds or target the same cellular processes as drugs currently in use. While analogs and derivatives are far quicker to develop, agents identified by this approach may have cross-resistance problem, as seen for the new rifamycins or quinolones [55].Approaches and novel sets of microbial targets need to be taken in consideration. One example of a promising new compound is diarylquinoline TMC-207 which acts through a novel molecular mechanism, most probably by inhibiting the ATPase synthase, leading to ATP depletion and pH imbalance [56]. What also comes to light from a critical analysis of the drug pipeline is that rational

approaches are weakly implemented in anti-TB drug discovery and development. Even the most promising novel drug candidates currently in clinical stage were identified serendipitously in screenings that were not designed originally for activity against *Mtb*. Moreover these compounds were selected for their ability to kill actively growing bacteria. There is consensus among the TB community that in order to obtain a real breakthrough in TB therapy and drastically shorten the treatment, there is an urgent need to identify compounds acting on key targets that are essential for mycobacterial persistence. There is a growing awareness that different subpopulations of bacteria that vary for their metabolism and growing rate can co-exist in an infected patient. Novel and more effective drugs should be rationally designed to interfere with metabolic and physiological strategies used by the bacteria to survive to host immune defences. An example is the search for inhibitors of the isocitratelyase, an enzyme that has been proven to be involved in the "dormancy" response: compounds able to inhibit this enzyme are expected to kill persistent bacteria. However, most of the compounds in the current pipeline target actively growing bacteria and so have bactericidal but not sterilizing activity. Therefore, while drugs currently in the pipeline could significantly shorten the treatment to two to three months they are unlikely to lead to a major breakthrough and reduce the treatment to a matter of weeks or days.A critical obstacle to such rational design is the lack of a comprehensive characterization of the fundamental biology of mycobacteria as they persist in human tissues. Thus, the identification and validation of potential targets that are relevant for the survival of the bacteria in vivo still represent a difficult task. This high degree of uncertainty about biochemical processes and molecular targets that can be potential target for effective new drugs renders the whole drug research and development process risky and, therefore, even less attractive for pharma investments. There is urgent need for a better characterization of heterogeneity of TB lesions to obtain a clearer picture of the different microenvironments in which *Mtb* persists. Moreover, there is still a pressing need to decrease the degree of uncertainty about the critical metabolic processes that drugs should target to achieve sterile mycobacterial elimination. The molecular pathways of persistence, identifying novel targets and subsequently run target-based drug discovery programs. A second critical obstacle to the implementation of rational drug design is the lack of well-validated drug targets. The fundamental genetics of *Mtb* growth and persistence in animal models are slowly being unraveled. Several enzymes involved in alternative metabolic pathways, energy generation, micronutrient acquisition, and survival in activated macrophages as well as in patient lesions have recently been identified as new sets of potential anti-microbial targets [57-65], but validation of these potential targets through genetic or chemical inactivation is largely missing. There is an urgent need to translate this advanced knowledge about *Mtb* metabolism and physiology into validated targets that can be used for screening of new lead compounds. A key difficulty lies in securing sustained funding for research projects that fall into the area of target validation and chemical genetics. The drug discovery and development when it comes to drugs for diseases like TB.

Conclusion:

Drug resistance in *M. tuberculosis* occurs by random spontaneous chromosomal mutations during natural cell replication. These mutations are not drug induced and are not linked. The probability of a drug resistance mutant occurring is directly proportional to the size of the bacterial population. The probability of spontaneous mutants being simultaneously resistant to two or more drugs is the product of the individual mutants. The development of drug resistance is a man-made amplification of a naturally occurring phenomenon. Previous treatment for TB predisposes to the selection of MDR organisms. Non-compliance is a major factor in allowing the resistant organisms to survive.

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