Biochemistry & Pharmacology: Open Access

Amaral and Soolingen, Biochem Pharmacol (Los Angel) 2014, 3:6
DOI: 10.4172/2167-0501.1000148

Commentary Article Open Access

Advances in Personalised Treatment of Multi-drug Resistant Tuberculosis

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Introduction

Pulmonary tuberculosis, an infection that has plagued man since the onset of civilization [1] and once effectively treated with isoniazid (INH) and rifampicin (RIF), has become a problematic disease, which is hard to cure due to the emergence of multi-drug resistant *Mycobacterium tuberculosis* (Mtb). This increasing problem is the consequence of ineffective prescription, non-compliance and transmission of resistant bacteria [2]. Multi-drug resistant tuberculosis (MDR-TB) is defined as resistance to isoniazid (INH) and rifampicin (Rif) and under strict conditions including monitoring of patient compliance, it may be successfully treated with a combination therapy of 5 or more drugs [3]. However, MDR-TB in recent years has progressed to resistance to more and more drugs [4]. Extensively drug resistant tuberculosis (XDR-TB) includes, apart from resistance to INH and rifampicin, also insensitivity to other first- and second line drugs (at least one injectable antituberculosis drug and any fluoroquinolone). More recently, the unofficial term "totally drug resistant tuberculosis" (TDR -TB) has been introduced which defines resistance to all available and approved anti-tuberculosis drugs [5]. MDR, XDR and TDR-TB continue to increase in frequency in indigent countries and to a lesser extent in economically advantageous countries [6].

Proposed Role of Personalized Clinical Laboratory Service for full evaluation of MDR, XDR and TDR *Mtb* strains that make it Possible to Select Effective Therapy

General approach

Control of tuberculosis lies primarily in early case finding, prevention of transmission and successful therapy.Adequate therapy requires monitoring of patient compliance by programmes that directly observe intake of medication as prescribed (DOTS) [7] and foremost that the selected therapy is effective. The prediction of effectiveness of the therapy depends onaccurate characterization of the causative mycobacteria. This includes the identification of the causative *Mycobacterium* at the genetic level, which immediately provides information relevant to the pathogenicity of the infective agent and the necessity of treatment. This identification can nowadays often be done directly on mycobacteria present in clinical material such as sputum, but in some cases has to wait for the isolation on culture medium. Identification of *M. tuberculosis* always warrants treatment, whereas finding nontuberculous mycobacteria (NTM) in some instance requires prescription of drugs. Next to DNA sequencing, several reversed line blot technologies are available, such as the ones of the Hain Company (Nehren, Germany). Another example of a laboratory assay that can simultaneously identify and type *M. tuberculosis*on genotype family level is spoligotyping, which has been utilized successfully at the global level by NalinRastogi [8]. Rapid indicative determination of resistance to INH, Rif, and certain second line drugs has become reliable and should, after the laboratory diagnosis has been established, directly steer the choice of the treatment regimen. The determination of resistance to Rif is afforded by the identification of mutations within the beta subunit of the *rpoB* gene [9]. Because resistance to Rif is almost always accompanied by resistance to INH [10], resistance to Rif acts as a surrogate marker for identification of MDR-TB [10]. The presumptive identification of MDR-TB can be done within a single day [10] and this therefore can rapidly identify patients in the need of intensified therapy, whilst pan-susceptible infections can readily be managed with the four first line drugs recommended by the WHO. It should be stated that in some geographic areas with a high prevalence of MDR-TB, each case of tuberculosis is nowadays treated more intensively with second line drugs [11]. Unfortunately, this more aggressive therapy produces a high degree of morbidity [12], and patient non-compliance occurs with high frequency due to its toxicity [13]. Furthermore, the accurate assessment of resistance to second line drugs has remained difficult given that the break points have not been fully established for each of the second line drugs and the critical concentrations are sometimes not well separated from frequently found MICs among susceptible strain populations.

Additional Laboratory Procedures that define cause, partly or wholly, for mdr phenotype: over-expressed efflux pumps

The above described laboratory diagnostic process is generally accepted. However, to this well-known battery of laboratory procedures, we may consider to add the determination of the over-expression of efflux pumps, which plays an important role in the development of resistance, especially in particular successfully spreading MDR-TB strains. Efflux pumps, which lower the concentration of toxic drugs in the intracellular environment, may be up-regulated and contribute to the survival of infective bacteria. The over-expression of efflux pumps in fact protects the infective Mtb from two or more anti-tuberculosis drugs by extruding these agents before they reach their intended targets [14]. Generally, the first response to a noxious agent such as an anti-tuberculosis agent, will result in over-expressed efflux pumps in the concerned bacteria [14-16]. With prolonged exposure of Mtb to the noxious agent, mutations are accumulated in key targets of the applied drugs in the surviving bacteria [17,18] and later, when the expression of the efflux pump(s) returns to base-line levels results in resistance to two or more antibiotics [17]. Consequently, next to the determination of resistance mutations in the known resistance genes, it is conceivable the (potential) multi-drug resistant phenotype is only fully characterized by measuring the activity of the efflux pump system in the Mtb isolate, although this is not yet a common practice at all. Nor is it known what

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Received: August 22, 2014; **Accepted:** September 26, 2014; **Published** September 30, 2014

Citation: Amaral L, Soolingen D (2014) Advances in Personalised Treatment of Multi-drug Resistant Tuberculosis. Biochem Pharmacol (Los Angel) 3: 148. doi:10.4172/2167-0501.1000148

the absolute role of efflux pumps is in the development of resistance and whether this fluctuates among different types of strains or genotypes. Regular measurement of efflux pump activity in *M. tuberculosis* isolates in some innovative laboratories would fill this gap of knowledge.

If indeed up-regulation of efflux pumps in MDR/XDR and TDR-TB is identified, this process can be neutralized by drugs that inhibit the efflux pump expression in bacteria [19]. Moreover, some of these drugs also inhibit the efflux pumps of the macrophage that transport calcium and potassium ions [20], which are reagents necessary for activating the killing machinery of the phagolysosome in which the Mtb is trapped. As an example, the phenothiazine thioridazine (TZ), used as a neuroleptic in humans, inhibits efflux pumps of Mtb [20] as well as the $Ca^{++}$and K^{+}pumps of the macrophage [21] and at relevant clinical drug concentrations enhances the killing activity of the human macrophage against phagocytosed Mtb [21]. Because TZ alone [22] and in combination with anti-TB drugs inhibits the *in vitro* replication of Mtb [23], Abbate and his group successfully treated 18 XDR-TB patients with a combination of TZ and antibiotics to which the infective strains were initially resistant to [24]. That TZ alone and in combination with anti-TB drugs cures MDR-TB infections has also been confirmed in murine models [25-28]. Consequently, it is highly conceivable that after laboratory data have suggested that over-expressed efflux pumps constitute a major contribution to the MDR phenotype of causative Mtb, TZ can be considered a highly important addition to antibiotics, which can optimize the effectiveness of therapy of MDR/XDR and TDR-TB infections significantly. It is therefore time to explore the application of efflux pump inhibitors more extensively and in a structured manner.

Defining the activity of selected antibiotics on the phagocytosed infecting MDR *Mtb* strain: The *ex vivo* laboratory procedure.

Despite the fact that in many settings drug susceptibility testing of *M. tuberculosis* isolates is nowadays performed routinely, this does not prevent treatment failure and frequent relapse after presumed curative treatment. The reasons for this are not fully understood, but may be the result of differences in the penetration of drugs into the tissues of individual patients. Therefore, another attempt to optimize the laboratory diagnosis regarding the effectiveness of selected therapy may involve testing the susceptibility of the Mtb to particular antibiotics, after phagocytosis by the patient's own macrophage [21,29-31]. After all, the key to effective therapy in the majority of patients lies in the ability of the agent to reach the Mtb which is imprisoned in the phagolysosome of the pulmonary macrophage. Hundreds of agents are claimed to inhibit the replication of Mtb, but only a precious few can effectively enter the intracellular environment where the infective agent commonly resides; generally the pulmonary macrophage. If a laboratory test would evaluate the *ex vivo* effectiveness of drugs to kill intracellular Mtb in the macrophages of the concerned patient, this would provide important information in addition to that from *in vitro* susceptibility assays to support the selection of therapy and strengthen the confidence in the selected approach. Again this could be tested in a selection of advanced laboratories in a pilot study.

Conclusion

In conclusion, therapy of tuberculosis should be "tailor made" for each patient in order to be effective and should be based upon extensive characterization of the causative Mtb. Not only drug susceptibility of Mtb isolates, but also the penetration of the drugs in macrophages is of crucial importance and testing this would most likely optimize the laboratory diagnosis significantly. Apart from this, there is still little attention at diagnostic laboratories for a general resistance mechanism in Mtb involving up-regulation of efflux pumps, while treatment to down regulate this form of (pre) resistance seems to contribute to *in vivo* treatment success. Therefore, clinical mycobacteriology laboratories are urged to consider extending the personalised laboratory diagnostic algorithms in their routine activity. After a pilot phase in advanced laboratories, practical approaches can be worked out to test the efflux pump activity of Mtb isolates and penetration of drugs in the macrophages of the concerned TB patient most efficiently. Lastly, it is important to note that proposed scheme of personalized laboratory service is highly cost effective. As treatment ofMDR-TB, and especially XDR-TB is extremely expensive, costing on the average half a million USA dollars per case of MDR TB in the USA [32], the proposed more extensive laboratory proceduresthat prevent multiplication of resistance are highly cost-effective.

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