SOLVING THE DILEMMA
OF ANTIMARYCOBACTERIAL CHEMOTHERAPY

Organized by N. Rastogi and J.O. Falkingham, III

Introduction

N. Rastogi (1) and J.O. Falkingham, III (2)

(1) Institut Pasteur de la Guadeloupe, Tuberculosis and Mycobacteria Unit, B.P. 484, 97165 Pointe-a-Pitre Cedex, Guadeloupe (French West Indies), and
(2) Department of Biology, Virginia Polytechnic Institute, Blacksburg, VA 24061-0406 (USA)

The increased prevalence of tuberculosis (i.e. Mycobacterium tuberculosis) and of infections in AIDS patients caused by non-tuberculous mycobacteria, especially by members of the Mycobacterium avium complex (i.e. M. avium, M. intracellulare, and M. species X), has made evident long-standing problems in the chemotherapy of these mycobacteria. These problems include: (1) inability to rapidly provide knowledge of drug susceptibility or resistance, (2) lack of success in chemotherapy and (3) lack of correlation between laboratory assessment of susceptibility or resistance and successful or unsuccessful response to therapy. Some of those problems are due to the fact that non-tuberculous mycobacteria are intracellular parasites, and thus in vitro susceptibilities may not serve as useful guides for treatment of infected patients. Additionally, evidence is now accumulating that a substantial proportion of AIDS patients are infected with more than a single M. avium complex strain (i.e. polyclonal infection). However, those problems notwithstanding, there has simply been a lack of knowledge about the mechanisms of action of antimycobacterial drugs and the mechanisms of resistance. In addition, there exists a need to identify potential targets for antimycobacterial chemotherapy and develop new and effective antimycobacterial drugs and chemotherapeutic regimens. It is the objective of this Forum to present the "state of the art" with respect to antimycobacterial chemotherapy, to provide a review and, more importantly, to offer a challenge.

The contributions in this Forum point up the strength of a multifaceted experimental approach. The mechanism of action of several antimycobacterial drugs has now been established by a combination of three approaches: characterization of the physiologic response of mycobacteria to antibiotic challenge; characterization of antibiotic-resistant mutants; and identification of genes for antibiotic targets by molecular genetic approaches. A case in point is the emerging picture of the action of isoniazid and the basis of mycobacterial resistance. First, a series of studies demonstrated the role of peroxidase-mediated isoniazid oxidation (Shoeb et al., 1985a) and of the generation of active oxygen metabolites in its activity (Shoeb et al., 1985b). Sec-
ond, those results were consistent with observations that a proportion of isoniazid-resistant mutants of \textit{M. tuberculosis} were shown to be catalase-deficient (Subbaiah et al., 1960; Mitchison et al., 1963; Jackett et al., 1978). Third, molecular genetic approaches confirmed that isoniazid-resistant strains of \textit{M. tuberculosis} had mutations in the catalase-peroxidase gene (Zhang et al., 1992; Heym et al., 1993; Morris et al., 1995). Finally, demonstration that the introduction of the wild-type catalase-peroxidase gene (\textit{katG}) into isoniazid-resistant mutants of \textit{M. tuberculosis} restored isoniazid sensitivity (Zhang et al., 1993) proved that isoniazid activity in mycobacteria requires oxidation by the catalase-peroxidase. In a similar fashion, the recent cloning and sequencing of another \textit{M. tuberculosis} gene responsible for conferring isoniazid resistance (i.e. \textit{inhA} gene) led to its identification as an enoyl-acyl-carrier protein reductase (Banerjee et al., 1994; Dessen et al., 1995). That finding is consistent with earlier physiological observations that isoniazid interfered with mycolic acid synthesis (Winder, 1982). Thus, it is expected that a combination of approaches will be required to identify the mechanism of action of antimycobacterial drugs.

Identification of drug-resistance mutations has been made possible employing the polymerase chain reaction (PCR). Because of the conservation of those genes whose products are the targets for the action of such antibiotics as rifampin (Telenti et al., 1993a; Williams et al., 1994), fluoroquinolones (Takiff et al., 1994; Cambau et al., 1994), streptomycin (Nair et al., 1993; Honore and Cole, 1994), and clarithromycin (Meier et al., 1994), PCR amplification of mycobacterial target gene sequences has been possible. Further, there appears to be a conservation of mutations leading to resistance (Telenti et al., 1993a; Honore and Cole, 1994; Meier et al., 1994; Williams et al., 1994; Morris et al., 1995). Thus, amplification can be targeted to those sequences which lead to resistance in other bacteria. One of the interesting future consequences of such studies will be the comparison of the spectrum of mutations represented by antibiotic-resistant mutants derived in the laboratory with those isolated from patients. It will be valuable to determine whether the mutations found in patient isolates represent a subset of those isolated in the laboratory, as appears to be the case amongst isoniazid-resistant mutants of \textit{M. tuberculosis} (Rouse and Morris, 1995). Existence of such a subset amongst resistant clinical isolates suggests that there exists selection against certain mutations.

The ability to amplify specific mycobacterial sequences has, in turn, given rise to the development of methods for the rapid identification of drug-resistance mutations in clinical isolates, without recourse to measurement of susceptibility. This approach involves the amplification of sequences known to lead to antibiotic resistance (Telenti et al., 1993b), and requires knowledge of the full spectrum of mutations leading to resistance to each antibiotic that could conceivably be used. Thus, there is a need to exhaustively identify all mutations leading to drug resistance if this approach is to be successfully employed.

Aside from identifying antibiotic resistance mutations, methods to rapidly assess mycobacterial susceptibility towards antibiotics are being developed. One of the more intriguing approaches has involved the use of the luciferase gene. In one application, the bioluminescence or luciferase genes (\textit{lux}) are used as reporters of the ability of a plasmid (Andrew and Roberts, 1993; Cooksey et al., 1993) or bacteriophage (Jacobs et al., 1993) to replicate in the cytoplasm of a mycobacterial cell. If an antibiotic is active, plasmid or phage replication and gene expression is reduced. By contrast, if the antibiotic is inactive because the cell is resistant, plasmid replication and gene expression, which includes the \textit{lux} gene, continues. The attractiveness of such a technique is that the sensitivity of detecting luciferase-catalysed light emission allows the rapid detection of antibiotic resistance.

A number of the contributions in this Forum establish the validity of rational approaches for antibiographic design and the identification of novel targets. These studies involve analysis of structure-function relationships and can identify new directions for antibiographic development (Klopman et al., 1993). These studies will supplement those of the past, which demonstrated increased antimycobacterial activity of hydrophobic derivatives of standard antibiotics (Sanfilippo et al., 1980; Rastogi et al., 1988). That development took advantage of knowledge of the extreme hydrophobicity of most mycobacteria.

Studies of the mode of action of isoniazid and clofazimine suggest that oxygen metabolism of mycobacteria may become an important and useful target for antimycobacterial chemotherapy. Those studies have identified the critical role of the catalase-peroxidase in drug action. For isoniazid, the catalase-peroxidase is required for "activation" of isoniazid (Shoeb et al., 1985a; 1985b; Zhang et al., 1993). For clofazimine, a phenazine, catalase-peroxidase activity may protect against clofazimine activity (Warek and Falkingham, this Forum). It will be of interest to see whether isoniazid-resistant mutants (resistant by virtue of \textit{katG} mutations) are more susceptible to clofazimine and whether any clofazimine-resistant mutants which overproduce the catalase-peroxidase activity are hypersensitive to isoniazid.

The suggested possible interaction between isoniazid and clofazimine is just one example of a powerful approach to antimycobacterial chemotherapy:
synergism. It is well documented that combinations of antimycobacterial drugs involving ethambutol (Hoffner et al., 1989) or other inhibitors of cell wall synthesis (Rastogi et al., 1990) take advantage of the antibiotic's ability to decrease the permeability barrier of mycobacteria. It will be important, in the future, to develop a rational approach to the identification of synergistic antimycobacterial combinations. For example, it is possible that the slow growth rate of most mycobacteria allows them to better survive the initial challenge of antibiotic. Survival may be enhanced through the induction of cell activities which protect cells against antibiotic challenge. Protective activities could include the carotenoid pigments, which are capable of scavenging oxygen radicals (Burton and Ingold, 1984), and enzymes (e.g. catalase) to degrade toxic oxygen metabolites. If survival of mycobacteria following such exposure to antibiotics did involve induction of protective activities, combinations which include inhibitors of mRNA (e.g. rifampin) or protein synthesis (e.g. streptomycin) should demonstrate synergism.

The organizers of this Forum hope that the array of articles will not only provide a timely and up-to-date review of the status of antimycobacterial chemotherapy, but will stimulate further research.

References


The basis of tuberculosis treatment

Tuberculosis is localized primarily in the lung, the main lesion being the pulmonary cavity, which contains about \(10^8\) mycobacteria \((= 10^8 \text{ cfu})\) (Canetti, 1959). Among these organisms, which are normally drug-sensitive, there are drug-resistant mutants at a mean frequency of \(= 10^{-6}\) (Canetti & Grosset, 1961). In the pulmonary cavity, a large bacillary population is located in the thin liquid caseous layer that covers the inner part of the cavity wall. These bacilli are extracellular and multiply actively because of the favourable oxygen tension and nutritive substances found in the cavity. In addition to the large population in the cavity, there are at least two other bacillary populations, one inside macrophages and another inside solid caseous foci. Both of these populations are limited in size because environmental conditions are unfavourable for growth (Grosset, 1980).

In the mid-1940s, when streptomycin (SM) was first introduced as chemotherapy for cavitary pulmonary tuberculosis, it was administered alone, with the result that, in 3 months' time, 80% of patients were harbouring streptomycin-resistant organisms (Medical Research Council Investigation, 1948). The explanation of such a phenomenon is simple: before treatment \(= 10^1-10^2\) streptomycin-resistant mutants were already present in the tuberculous cavity along with the \(10^8\) sensitive organisms, and these mutants were selected by the use of streptomycin.

Soon it was understood that there was no hope of curing cavitary tuberculosis with any single antimicrobial drug because it was impossible to prevent the selection of drug-resistant mutants under these