Treatment of mycobacterial infections

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Summary

Treatment of mycobacterial infections differs from that of other bacterial diseases due to several properties possessed by the mycobacteria and the host. A hallmark of mycobacteria is the complex lipid-rich cell envelope that protects the organism from both the host response and antimycobacterial therapy. In addition, mycobacteria are facultative intracellular parasites which generally cause a more chronic type of disease. These properties add greater constraints to efficient therapy. To be effective, drugs must be able to penetrate the host macrophage and preferably have reduced toxicity and be effective at low doses to allow prolonged therapy. The author presents the general properties of the pathogen/host relationship in mycobacterial infections, in addition to the therapeutic choices available and the mechanisms of action involved in treatment. The evolution of technology for antimycobacterial therapy is illustrated by a discussion of new strategies being developed for the treatment of mycobacterial infections.

Keywords


Introduction

Mycobacterial infections represent some of the most difficult diseases to treat in humans and animals. Because of the high lipid content and complexity of the cell envelope, mycobacteria are refractory to most antimicrobial agents (6, 88). In addition, mycobacteria are facultative intracellular parasites, capable of surviving and persisting within the host macrophage (6). This is the very cell that is largely responsible for eliminating pathogenic microbes. Given this intracellular infestation, agents that are to be effective in controlling and/or killing the pathogen must also be able to penetrate the host cell that harbours the mycobacteria. Intracellular effectiveness is therefore an additional attribute necessary for adequate antimycobacterial therapy. Initial screening of potential antimycobacterial agents should, therefore, also include appropriate experimentation to determine intracellular efficacy.

In addition to intracellular survival, some mycobacteria (e.g. Mycobacterium tuberculosis) are capable of persisting in the host tissue in a state of dormant existence referred to as latency. Although information about the latent state is limited, it is rationalised that the organism persists in an altered metabolic state, probably relying upon enzymatic pathways not used (or used only to a limited degree) during a normal life-cycle in the host. This would suggest possible new drug targets that could be included in future drug discovery efforts.

The chronic nature of mycobacterial infections generally necessitates prolonged therapy over several months. An ideal agent should therefore have low toxicity and be effective at low dose levels. The importance of these considerations is further amplified because mycobacterial infections are treated with multiple drug regimens (116, 125). Multiple drug regimens are necessary to prevent the development of drug resistant strains, an event that occurs at different frequencies depending upon which drug is considered. However, the use of multiple drugs increases the likelihood of toxic side-effects that may influence therapeutic success.

The purpose of this paper is to review the various antimicrobics that are used to treat mycobacterial infections. In general, the same agents are used to treat mycobacterial
infections in both animals and humans. Information in the published literature is limited with regard to dosing and therapeutic regimens for various animal species. In some cases, animals are euthanised rather than being treated. However, chemotherapy has been reported to be successful in treating cases of tuberculosis in monkey colonies. In one case, rhesus monkeys (Macaca mulatta) received isoniazid, rifampin and ethambutol once daily for twelve months, with the conclusion that chemotherapy could provide an alternative to destruction of the animals (125). In another case, an epidemic of tuberculosis occurred in a non-human primate breeding colony containing cynomolgus monkeys (Macaca fascicularis) and rhesus monkeys (116). The animals were treated with a combination of streptomycin and isoniazid, with similar conclusions to the former study, namely that chemotherapy was a viable alternative to euthanasia (116).

Dosing and treatment schedules vary and these will not be discussed in this paper. Instead, each of the agents will be described in terms of mode of action and in vitro and in vivo efficacy. In addition, the development of new antimycobacterial agents will be discussed with special emphasis on drug discovery and design, in addition to delivery systems being developed for improved treatment of mycobacterial infections. The aim is to present the relevant information to enable the reader to analyse existing data effectively and derive a personal evaluation of potentially useful antimycobacterials for use in a specific area of therapy.

Pathogenesis

Intracellular parasitism

As stated earlier, mycobacteria are facultative intracellular parasites (6). Unlike obligate intracellular parasites (e.g. viruses, chlamydia, rickettsia, etc.) which are completely dependent upon an intracellular environment, mycobacteria are capable of existing both intracellularly and extracellularly within the infected host. This is extremely important from a therapeutic standpoint because effective therapy requires another level of competency which is not necessary for strictly extracellular parasites. To be effective against mycobacteria, drugs must also be able to penetrate host macrophages, in addition to the cell envelope of the pathogenic organism. Testing for antimycobacterial agents must therefore include efficacy studies involving macrophages. Generally, this type of testing can be grouped into one of two categories. Some investigators utilise macrophages derived from animals, for example, peritoneal, bone marrow or alveolar macrophages. The other category of testing involves cell lines. One of the most commonly-used animal cell lines is the murine J774, an adherent macrophage cell line that has been used extensively for antimycobacterial drug screening.

Evaluation of intracellular efficacy requires the initial infection of macrophages with the mycobacterium in question (126). The mycobacterium should be able to replicate intracellularly within the time frame of the experimental protocol (generally from five to seven days) and produce a significant increase in colony forming units (CFUs) which can be used for statistical analysis to compare the treated and control groups (126). Toxicity evaluations should be included to verify that inhibition of growth is due to the drug under evaluation and not the result of lysing macrophages that may falsely indicate positive drug action. For example, some mycobacteria may not replicate in the tissue culture medium after being released from lysed macrophages. This would result in an apparent decrease in CFUs when compared to the non-treated control, and hence an incorrect evaluation of the drug being tested.

While not all species of mycobacteria have been tested using these systems, it is very likely that any mycobacterial species can be utilised, providing that the investigator establishes the necessary parameters for the species in question. Thus, antimycobacterial drug testing can potentially be performed with any mycobacterium. It is also very likely that other animal macrophage systems could be developed to evaluate potential antimycobacterial action. Ideally, intracellular efficacy evaluations would be performed with macrophages derived from the species to be treated with the drug. As this may not always be possible, commonly-used systems, such as the J774 murine cell line, should be adequate if development of appropriate animal macrophage systems is not feasible (6, 35, 82, 89, 126, 127). Hence, the development of other macrophage assays could potentially be very useful, but the use of established procedures should not be precluded.

Another important aspect of intracellular efficacy evaluations for antimycobacterial drugs involves the variation of environmental parameters found within the host and host macrophages. Although exact parameters may not have been established for this intracellular environment, it has generally been observed that growth of mycobacteria in a host is quite different from that observed in vitro (123). This difference is due in part to the choice of substrates available to the infecting mycobacteria. This is important because a drug target (e.g. an enzyme in a key pathway) that may be upregulated in vitro may not necessarily be present at the same level when the mycobacteria grow intracellularly. It is possible that the drug target may not be expressed at all in the intracellular growth pattern. If that is the case, the potential drug would not be expected to produce the optimum effect when used to treat the animal in question.

Thus, intracellular efficacy evaluations are an important part of the development and screening of any antimycobacterial drug. Although this type of testing may be tedious and require a different level of expertise, the process is essential to properly evaluate the therapeutic potential of antimycobacterial drugs.
Dormancy (latency)

As mentioned in the Introduction, the ability of mycobacteria to persist in a dormant state is also an important consideration for optimum mycobacterial therapy. In this discussion, M. tuberculosis will be used as the representative organism. Studies involving specific aspects of dormancy are limited (20, 120). However, evidence from an in vitro system suggests that various stages occur in the 'shiftdown' and 'shiftup' of M. tuberculosis as the organism enters and exits dormancy (117, 119). During the 'shiftdown', the organism apparently goes through two or more stages. In the first stage, the organism shifts from rapid to slow replication (117). The second stage involves a complete shutdown of replication without death of the organism. Three markers have been described in this process, as follows:

- **a)** changes in tolerance to anaerobiosis
- **b)** production of a unique antigen
- **c)** an increase in glycine dehydrogenase production (117, 118, 121).

During the 'shiftup' process, initial production of ribonucleic acid (RNA) occurs, followed by the initiation of deoxyribonucleic acid (DNA) synthesis (119), indicating that during dormancy, the metabolic events that occur serve a completely different purpose. This would suggest that targets for optimal therapy would be different from those found in an active disease state and would therefore require different antimicrobics and therapeutic regimens (122). Investigations of dormancy are currently in progress and hopefully a more specific evaluation will eventually result. However, an awareness of this variation in metabolism is important because it most certainly indicates a different assessment for therapy.

Antimycobacterial agents

Antimycobacterial agents will be discussed as two major groups. The first group consists of antimycobacterial agents that require some type of activation by the mycobacteria. The term used to describe this type of compound is 'prodrug'. A prodrug is a compound that is converted to an active derivative by biotransformation or non-enzymatic processes such as hydrolysis, oxidation or reduction (7). Generally, these compounds are more selective for mycobacteria because the targets are associated with mycobacteria and not other microorganisms. These targets are generally involved in the synthesis of unique structures found in the cell envelope of mycobacteria, including mycolic acid, arabinogalactan and lipoarabinomannan. The second group consists of antimycobacteria that do not require activation by mycobacteria and have a broader spectrum of activity. This is due to the fact that the targets of these drugs are found in other bacteria as well as mycobacteria. These compounds affect a specific target, such as an enzyme in a key pathway or a subunit in the bacterial ribosome.

Antimycobacterials requiring activation by mycobacteria

Isoniazid

Isoniazid (isonicotinic acid hydrazide [INH]) is an analogue of nicotinamide and, amongst various mycobacteria, is most effective against M. tuberculosis. This is a very potent and specific drug for M. tuberculosis, with relatively low toxicity (56). Early studies described the ability of INH to inhibit mycolic acid synthesis and the hypothesis which developed suggested this mechanism as the primary cause of mycobacterial cell death (56, 124). Later work by Takayama and associates (99) elucidated mycolic acid metabolism and demonstrated that INH inhibits the desaturation of C24 and C26 acids, thus leading to an accumulation of saturated fatty acids. More recent studies have shown that resistance to INH is associated with mutations of the inhA gene (3, 18, 76, 130). Isonicotinic acid hydrazide is one of several antimycobacterial agents that requires activation for effectiveness. Catalase-peroxidase activity, encoded by the katG gene (32, 71, 72, 77, 78), is responsible for oxidation of INH which eventually results in toxicity to M. tuberculosis, after several intermediary steps. Despite the wealth of information that has been obtained, the precise target for the action of INH remains unknown (3). Although INH is most effective against M. tuberculosis, some activity is apparent against a few non-tuberculous mycobacteria, in particular M. kansasii, but not against M. avium (24).

Ethambutol

Ethambutol (EMB) is a synthetic drug that was developed from N,N'-diisopropylethylenediamine and has been demonstrated to have activity against M. tuberculosis (104). The drug is active against M. tuberculosis, M. kansasii and some other non-tuberculous mycobacteria, including M. avium (24). Ethambutol prevents the polymerisation of cell wall arabinan by inhibiting the introduction of D-arabinose into arabinogalactan and lipoarabinomannan (60, 61, 75, 100, 101).

Pyrzaminamide

Pyrzaminamide (PZA) is the amide of pyrazinoic acid that is metabolised to 5-hydroxypyrazinamide after absorption from the gastrointestinal tract (56). Pyrazinamide is active against M. tuberculosis, but not other members of the M. tuberculosis complex (e.g. M. bovis), nor non-tuberculous mycobacteria such as M. avium (24, 53, 56). Although the exact mechanism of action of PZA is not known, studies have shown that PZA is converted to pyrazinamide by nicotinamide (63). One hypothesis suggests that PZA functions as a prodrug of pyrazinamide (95). Additional studies have suggested that susceptibility of M. tuberculosis to PZA is due to a deficient pyrazinamide efflux mechanism, whereas resistance is due to a highly active efflux pump (131).
Aminoglycosides represent one of the oldest groups of antimycobacterials. These bactericidal agents are characterised by combinations of six-membered aminocyclitol rings with varying attached side chains. The aminoglycosides are broad spectrum antibiotics, having activity against both Gram-positive and Gram-negative bacteria, in addition to mycobacteria.

Macrolides

Macrolides are a group of broad-spectrum bacteriostatic antibiotics, some of which have activity against certain mycobacteria. The mechanism of action is inhibition of protein synthesis, which is accomplished by binding of the drug to the 50S ribosomal subunit (33). The macrolides include erythromycin, azithromycin, clarithromycin and roxithromycin.

The macrolides appear to have better activity against non-tuberculous infections, such as those caused by the M. avium complex (9, 10, 16, 52, 67, 102, 129), than against M. tuberculosis (110, 128). Reports indicate that clarithromycin also has activity against M. ulcerans (86) and M. marinum (14).

Fluoroquinolones

The fluoroquinolones are bacteriocyclic carbonic acid derivatives which are structurally related to nalidixic acid. Included in this group are ciprofloxacin, clinafloxacin, gatifloxacin, levofloxacin, ofloxacin, moxifloxacin, sitafloxin and sparfloxacin. The primary target for the quinolones is the bacterial DNA gyrase (topoisomerase II) (56). Binding of the drug to the enzyme prevents subsequent processes that depend upon the proper maintenance of DNA superhelical twists, such as replication and transcription (17).

Generally, the fluoroquinolones are active against several mycobacteria, including M. tuberculosis, M. bovis, M. fortuitum, M. kansasii, M. peregrinum and M. leprae (49, 57, 58, 107, 108, 111). These drugs are not very active against M. avium complex, M. intracellulare, M. marinum, M. chelonae and M. abscessus (49, 57, 111).

Oxazolidinones

Oxazolidinones are a relatively new group of totally synthetic antimicrobial compounds that were developed in the late 1970s by DuPont (1, 47). These compounds inhibit protein synthesis, presumably at a site that precedes chain elongation, or more specifically, before the interaction of fMet-transfer RNA (fMet) and 30S ribosomal subunits with the initiator codon (39, 40). Some of these agents have shown activity against M. tuberculosis in vitro (2, 4) and in a murine model (25). Further testing is needed to evaluate the full potential in treating mycobacterial infections and also toxicity.

Antimetabolites

Antimetabolites are a compound which is sufficiently similar in structure to a normal metabolite that it can occupy the enzyme binding site in place of the metabolite, thus inhibiting a biosynthetic pathway (94). Antifolates are a group of antimetabolites which have been utilised for antimicrobial drug therapy in the past. A target for this class of drugs is...
dihydrofolate reductase (DHFR), a key enzyme in the folate metabolic pathway which is necessary for the biosynthesis of RNA, DNA and protein. The enzyme is an important target for medicinal chemistry (13), and inhibitors of DHFR have been used in chemotherapy for cancer (methotrexate [11]), bacterial infection (trimethoprim [41]), and malaria (pyrimethamine [92]). All cells contain DHFR, and the activity of this enzyme is necessary for the maintenance of intracellular folate pools in a biochemically active reduced state (70). Inhibition of DHFR is effective because binding affinities for substrate analogues are so great that these analogues are not readily displaced by the natural substrates (70). Inhibition results in the depletion of intracellular reduced folates necessary for one-carbon transfer reactions which are important for biosynthesis of RNA, DNA and protein. Although some bacteria have an uptake system for folates, most have to synthesise folates de novo. Reduction of dihydrofolate to tetrahydrofolate is a universal requirement (50).

Investigations of the antimycobacterial properties of a group of antifolate compounds that are derivatives of 2,4-diamino-5-methyl-5-deazapteridine (DMDP) have been performed in the Mycobacteriology Research Unit at the Southern Research Institute, Alabama, United States of America (USA). These lipophilic compounds have structures similar to the piritrexim/trimetrexate class of antifolates and have demonstrated activity against _M. tuberculosis_ (96) and to an even greater extent, against _M. avium_ (96, 97). The _M. avium_ folA gene, which codes for DHFR, has been identified, cloned and expressed for use in enzyme assays designed for drug screening and identification (132, 133). Subsequently, the recombinant _M. avium_ DHFR was used to screen more than seventy DMDP derivatives and a structure/activity relationship was identified that resulted in the synthesis of a DMDP derivative with reduced toxicity for human DHFR, specificity for _M. avium_ DHFR, and intracellular efficacy in a _M. avium_-infected macrophage model (98). Other workers have shown an enthusiasm in the development of improved derivatives of this class of inhibitor with regard to mycobacteria (26, 59, 69, 74, 103). Further evaluation and testing will be necessary to develop more effective DMDP derivatives for clinical trials; these studies are in progress.

Macromolecular aspects of inhibition by antimycobacterial drugs

In this section, only those antimycobacterials that have a specific mechanism of action (i.e. those not requiring activation by the mycobacteria) will be discussed. The following examples are included to give a representation of the effects of certain antimycobacterials at the macromolecular level. These examples will include inhibition of protein synthesis (e.g. aminoglycosides), RNA synthesis (e.g. rifamycins) and DNA synthesis (e.g. fluoroquinolones). The experimental procedures are designed to determine the inhibitory activity of each drug by observing the incorporation of appropriate radiolabelled precursors into respective mycobacterial macromolecular components. Thus, for protein synthesis, incorporation of [14C]-phenylalanine will be assayed, whereas for RNA and DNA synthesis, incorporation of [14C]-uracil will be assayed.

The use of radiolabelled thymine or thymidine should be avoided in monitoring DNA synthesis in _M. tuberculosis_, because of the presence of thymidine phosphorylase which hydrolysates the substrate (12, 118). Use of thymidine will result in misleading incorporation data. It is therefore necessary to use radiolabelled uracil, which is incorporated into both RNA and DNA, and to distinguish between radiolabelled RNA and DNA by hydrolysing the RNA prior to DNA precipitation from the cells using potassium hydroxide (118). In the case of DNA, uracil is methylated prior to incorporation into the macromolecule (48), therefore uracil that is radiolabelled at the 5-position with [3H] should not be used because the label will be lost during the methylation process.

In the following examples, [2-14C]-uracil was used for incorporation into RNA and DNA. Additional parameters to be considered in these assays are a negative control (e.g. a polymyxin such as polymyxin B which inhibits membrane function), a solvent control (e.g. equivalent amount of solvent necessary for dissolving the drug being tested), and a positive control for the biosynthetic process to be tested (e.g. streptomycin for protein synthesis, rifamycin for RNA synthesis and ofloxacin for DNA synthesis). The assay, which is depicted in Figures 1, 2 and 3, involves a short time period (1 h-5 h). Appropriate drugs are added to an actively growing culture of _M. tuberculosis_ H37Rv (avirulent strain), following addition of the appropriate radiolabelled component. Samples of mycobacteria are taken at various time intervals and processed for radiolabel incorporation by precipitation with trichloroacetic acid, followed by measurement of radioactivity using a scintillation counter.

Protein synthesis

For the protein synthesis assay, streptomycin (an aminoglycoside) is used as the inhibitor of protein synthesis (i.e. positive control). As discussed above, aminoglycosides inhibit protein synthesis by binding to the bacterial 305 ribosomal subunit, thus preventing elongation of polypeptides. The radiolabel in this case is [14C]-phenylalanine.

As illustrated in Figures 1a and 1b, inhibition is not immediate, occurring in these examples at approximately 2 h post treatment. Since translation of the already attached messenger RNA (mRNA) will continue following attachment of the drug to the appropriate receptor on the 305 ribosomal subunit, the round of protein elongation in progress will proceed until completion. Once that round of translation has finished, protein synthesis by the affected ribosome will cease because binding is irreversible. By adjusting the
a) Inhibition of protein synthesis by streptomycin sulphate (SSO₄) at 1 µg/ml, 5 µg/ml and 10 µg/ml

b) Inhibition of protein synthesis by streptomycin sulphate (SSO₄), rifampin (RIF) and ofloxacin (OFL)

Fig. 1
Effects of inhibitors on protein synthesis in Mycobacterium tuberculosis H37Ra

*Mycobacterium tuberculosis* H37Ra was cultured in Middlebrook 7H9 broth (10% oleic acid-albumin-dextrose-catalase [OADC], 2% glycerol) until exponential phase was obtained. [*¹⁴C*-phenylalanine was added, followed by the respective inhibitors (time 0). Samples containing viable mycobacteria were removed and proteins precipitated with trichloracetic acid (TCA, 10%). Radioactivity, as determined using a scintillation counter, is expressed as counts per minute (CPM) concentrations of the drug, a dose response can be observed (Fig. 1a). The MIC of streptomycin for *M. tuberculosis* H37Ra is 0.5 µg/ml-1.0 µg/ml. In Figure 1b, the effect of streptomycin is compared to that of rifampin and ofloxacin. Rifampin has an immediate effect on protein synthesis, whereas ofloxacin acts more slowly (Fig. 1b). This is explained by the fact that rifampin inhibits RNA synthesis which is immediately necessary for protein synthesis. Ofloxacin inhibits DNA supercoiling which will eventually affect protein synthesis, but not in this time frame.

Ribonucleic acid synthesis

For the RNA synthesis assay, RIF (a rifamycin) is used as the inhibitor of RNA synthesis (i.e. positive control). As discussed above, rifamycins inhibit RNA synthesis by binding to the beta-subunit of the DNA-dependent RNA polymerase. The radiolabel used in this case is [*²⁻¹⁴C*]-uracil.

Inhibition of RNA synthesis by rifampin is apparent early in the process (Figs 2a and 2b), as expected by the rapid onset of action of the drug. Adjustment of drug concentration around

Fig. 2
Effects of inhibitors on ribonucleic acid (RNA) synthesis in Mycobacterium tuberculosis H37Ra

*Mycobacterium tuberculosis* H37Ra was cultured in Middlebrook 7H9 broth (10% oleic acid-albumin-dextrose-catalase [OADC], 2% glycerol) until exponential phase was obtained. [*²⁻¹⁴C*-uracil was added, followed by the respective inhibitors (time 0). Samples containing viable mycobacteria were removed and nucleic acids precipitated with trichloracetic acid (TCA, 10%). Radioactivity, as determined using a scintillation counter, is expressed as counts per minute (CPM)
a) Inhibition of DNA synthesis by ofloxacin (OFL) at 0.5 µg/ml, 2.5 µg/ml and 5.0 µg/ml

b) Inhibition of DNA synthesis by ofloxacin (OFL), streptomycin sulphate (SS0) and rifampin (RIF)

Fig. 3
Effects of inhibitors on deoxyribonucleic acid (DNA) synthesis in Mycobacterium tuberculosis H37Ra

*Mycobacterium tuberculosis* H37Ra was cultured in Middlebrook 7H9 broth (10% oleic acid-albumin-dextrose-catalase [OADC], 2% glycerol) until exponential phase was obtained. [2-14C]-uracil was added, followed by the respective inhibitors (time 0). Samples containing viable mycobacteria were removed and RNA was hydrolysed with potassium hydroxide (K0H), prior to DNA precipitation with trichloracetic acid (TCA, 10%), following removal of RNA by K0H. Radioactivity, as determined using a scintillation counter, is expressed as counts per minute (CPM)

Deoxyribonucleic acid synthesis

In the DNA synthesis assay, ofloxacin (a fluoroquinolone) was used as the inhibitor of DNA synthesis. As discussed above, fluoroquinolones bind to the bacterial DNA gyrase, thus preventing proper maintenance of DNA superhelical twists. Ultimately, this results in inhibition of replication and transcription. The radiolabel for these experiments was [2-14C]-uracil.

Action of ofloxacin on DNA synthesis is immediate, with loss of radiolabel incorporation being seen after one hour (Figs 3a and 3b). The MIC of ofloxacin for *M. tuberculosis* H37Ra is 0.5 µg/ml and the dose response for concentrations around this MIC is given in Figure 3a. Although this inhibition will ultimately affect other parameters such as RNA and protein synthesis, these effects are not observed within the time frame of these experiments (Figs 1b and 2b). Deoxyribonucleic acid synthesis is affected by both rifampin and streptomycin

Delivery systems

In recent years, techniques for improved delivery of antimicrobics have been developed. These techniques have been beneficial in the therapy of mycobacterial infections, primarily because the formulations are designed to facilitate phagocytic uptake by host macrophages. As mycobacteria are intracellular parasites, these techniques have proven useful in the therapy of certain mycobacterial diseases. Two formulations which have been used for delivery of antmycobacterial drugs are liposomes and microspheres.

Liposomes

Liposomes are small phospholipid vesicles which are composed of concentric lipid bilayers with alternating aqueous compartments. These artificial vesicles have permeability properties which are similar to biological membranes. Delivery of various antimicrobics has been reported for therapy of mice infected with *M. avium* (8, 23, 34, 35, 36, 43, 68, 79, 80, 85) and *M. tuberculosis* (28, 83, 115). Use of liposomes allows for delivery of drugs to macrophages, but allows no flexibility with regard to timing of drug release.

Microspheres

Microspheres are discrete particles, the biological agent can either be encapsulated within the microsphere or attached to the surface (87). Microspheres are generally formulated with polymers, such as poly(lactide-co-glycolide) (PLG), that allow significant flexibility with regard to programmed release (5, 87). Degradation of PLG microspheres results from hydrolysis and not enzymatic processes (105). These properties enable the formulation of microspheres that release a drug for a period of days or months (small microspheres: 1 µm-10 µm in diameter) or for a year or more (large microspheres: 10 µm-150 µm), which would not be possible with liposomes.
(87). An additional benefit of microspheres is the ability to administer a greater quantity of drug than liposomes, on a weight basis comparison (83, 87, 115).

The chemical formulation of microspheres (PLG) is based upon the formulation for synthetic sutures (45). The PLG and also its metabolic products, lactic acid and glycolic acid, are known to be biocompatible (112, 113, 114). Microsphere technology has been used for sustained delivery of a variety of substances, including antigens, steroids, peptides, proteins and antibiotics (21, 37, 38, 55, 90, 106).

The intracellular efficacy of small rifampin-loaded microsphere formulations has been demonstrated in the Mycobacteriology Research Unit at the Southern Research Institute. These formulations have been shown to be effective against *M. tuberculosis*-infected macrophages by significantly reducing levels of intracellularly replicating mycobacteria (5). The small microspheres are better at delivering effective doses of rifampin intracellularly than equivalent doses of free drug (5). Subsequently, the effective use of both small and large rifampin-loaded microspheres was demonstrated in treating *M. tuberculosis*-infected mice (87). The microsphere formulations, administered in one or two doses during a one-month period, were able to achieve a reduction in mycobacteria similar to that obtained with a daily drug regimen of rifampin equivalent to 10-20 mg/kg/day. Programmed release of rifampin was obtained throughout the twenty-six-day experimental period (87). The results suggest that these type of drug delivery formulations can be used for therapy of mycobacterial infections and will allow reduced dosing and targeted delivery to host macrophages (5, 87).

Use of microsphere technology for treatment of animal diseases is very feasible, particularly with regard to reducing dosing intervals and toxicity. Recent studies at the Southern Research Institute with non-human primates (cynomolgus monkeys of approximately 4 kg) have revealed that as much as 4 g of a small rifampin-loaded microsphere formulation (1 µm-10 µm in diameter, 5.8% [wt/wt] loading of rifampin) can be injected intravenously (2 g given twice at one week intervals) without adverse effects on the animal (D.C. Quenelle and W.W. Barrow, unpublished data). The small microsphere formulation demonstrated sustained programmed release of drug over thirty days. In some experiments, animals were treated with intravenous injections (4 g total) along with 2 g of a larger microsphere formulation injected subcutaneously (27 wt% loading of rifampin). In these experiments, release of rifampin was observed for up to forty-eight days without any adverse reactions observed in the animals (i.e. body weights, histopathology, etc.) and without any biologically significant changes in liver enzyme activity (i.e. >1.5-fold change for alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase). Bioassay results demonstrated blood levels, urine levels and more importantly, concentration of rifampin in various tissues (e.g. liver, spleen and lungs) (E.L.W. Barrow and W.W. Barrow, unpublished data). These preliminary studies suggest that a microsphere drug delivery protocol offers an economically feasible and effective approach for treating companion and domestic agriculture species. Animals would be handled much less frequently and the outlay for drugs would be decreased since required dosages would be considerably reduced. Hence, this approach could reduce significantly both the cost and stress of treatment. For food animals, however, the impact on the withholding period prior to using the animal products would have to be evaluated.

**New strategies for antimycobacterial drug development**

Traditionally, drug discovery has been primarily dependent upon the screening of large collections of previously synthesised chemical compounds or products occurring naturally (46). However, since the early 1990s, new and improved techniques have evolved that will increase the opportunities for new drug discovery and improve the ability to synthesise more selective and active compounds for treating mycobacterial infections. Some of the new technological platforms that are being developed are discussed below.

### Genomics

The sequencing of a number of bacterial genomes has been achieved and work on others is progressing. Given the numerous genomic projects currently underway, the development of new and better drugs should be possible for a number of pathogenic microorganisms, including *M. tuberculosis* (19). This is extremely important for *M. tuberculosis* because of the increasing numbers of drug resistant strains that are developing (64, 84). Although approximately 16% of the estimated 4,000 encoded proteins of *M. tuberculosis* have still not been identified, 40% have been associated with known biochemical functions (7, 19). As suggested by Barry *et al.* (7), mycobacterial research has now shifted 'from gene hunting to interpretation of the biology of the whole organism'. Given this expanding genetic knowledge base, it should be possible to utilise sophisticated technologies to identify new drug targets and design new and improved therapeutic regimens for mycobacterial infections.

One such technology that will be helpful in observing differential gene expression is the DNA-based microarray technique. This technology should allow the analysis of the differential metabolic patterns of mycobacteria in vivo and in vitro (7). This technology can also be used to predict mechanisms of action of drugs (7).
Proteomics

The next level of technological platforms being developed for proteomics, a term that was coined in 1944 by a postdoctoral researcher from Australia by the name of Marc Wilkins (44). The term proteomics refers to the proteins that are expressed by a genome (44). Unlike DNA and RNA arrays, proteomics addresses the final gene product, i.e. the protein. The benefit of this technology is that the major shortcoming of DNA chip technology (i.e. failure to consider pre-translational events and post-translational modifications of proteins) is overcome (44). In the case of antimycobacterial drug development, this has been shown to be critical. An example of this was recently presented by Barry et al. (7), in a discussion of the mechanism of INH action. A covalent complex of INH with two different proteins, AcpM and KasA had been described (73). Although both related proteins were transcriptionally up-regulated, the actual interaction between the proteins and INH would not have been revealed without a proteomic experiment (7).

Initially, the major tool used for proteomics was two-dimensional gel electrophoresis, a tedious and laborious technique which is time-consuming and is only useful for proteins that exist in fairly large proportions in the cell (44). However, in the last few years, instrumentation companies have been able to exploit the use of mass spectrometry (MS) for improved analysis of the protein arrays. In 1989, matrix-assisted laser desorption/ionisation (MALDI) was introduced, in addition to electrospray ionisation (ESI) (44). These two developments greatly expanded the range of proteins that could be analysed by MS (44). Subsequently, a more rapid method of identifying proteins from MS was introduced, namely: protein mass fingerprinting (44). Currently, companies are developing more sophisticated instrumentation to render proteomics more feasible and useful for drug discovery (44). Although this type of technology is fairly new, it holds great promise for future drug discovery and development.

Combinatorial chemistry and structure-activity relationship

Combinatorial chemistry and structure-activity relationship (SAR) represent two additional technological platforms that are useful for the discovery and design of new drugs. The role of combinatorial chemistry is to synthesise large 'libraries' of related compounds that can be assayed by means of a high-throughput screening technique, a process which has been of great benefit in the task of random searching for drug candidates (65). Generally, these libraries are based upon a lead compound which has shown in vitro activity against the organism in question (7). Subsequently, a more focused library is generated around the most active substructures, to improve binding efficiency (7). This approach significantly accelerates the process of synthesising new drug candidates (65).

Structure-activity relationship involves the use of crystallography and nuclear magnetic resonance (NMR) to assess the three-dimensional structure of the target protein to enable the production of inhibitors that are complementary to the binding site (66). With the information derived from these sources, improved binding affinities can be obtained, thus optimising activity for the target protein (e.g. an enzyme in a mycobacterial biosynthetic pathway), while decreasing selectivity for the similar host protein, if applicable. Ideally, a drug should have a perfect geometric fit of the ligand to the binding site. As binding affinity information is obtained, new drugs can be synthesised and tested in appropriate in vitro and in vivo assays. One of the failings of SAR is the inability of the procedure to predict bioavailability and metabolic stability (66), characteristics which have to be accomplished by appropriate animal studies.

Conclusion

A number of effective and reliable antimycobacterial drugs are currently available for treatment of several mycobacterial infections. The primary reason for the loss of efficacy of some of these drugs is the emergence of drug resistant strains. Development of resistance is an important variable to be considered in the eradication of mycobacterial disease and should be treated very seriously. However, the recent advances in genomics and the development of new and improved biotechnological platforms suggest a promising future with regard to development of effective drugs for control and/or eradication of mycobacterial diseases.

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Traitement des infections mycobactériennes

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Résumé
Le traitement des infections mycobactériennes diffère de celui appliqué à d'autres maladies bactériennes en raison de plusieurs propriétés spécifiques des mycobactéries et de leurs hôtes. L'un des traits distinctifs des mycobactéries est l'enveloppe cellulaire complexe et riche en lipides qui protège le micro-organisme contre la réponse de l'hôte et contre les produits thérapeutiques antimycobactériens. Par ailleurs, les mycobactéries sont des parasites intracellulaires facultatifs qui provoquent généralement un type de maladie plus chronique. Ces propriétés sont autant d'obstacles à une bonne thérapie. Pour être efficaces, les médicaments doivent pouvoir pénétrer dans les macrophages de l'hôte ; il est également souhaitable qu'ils soient peu toxiques et qu'ils soient actifs à de faibles doses afin de permettre une thérapie à long terme. L'auteur explique les propriétés générales de la relation agent pathogène/hôte lors des infections mycobactériennes, et examine les différentes options thérapeutiques ainsi que les mécanismes sur lesquels repose le traitement. L'évolution récente des techniques thérapeutiques antimycobactériennes est illustrée par une description des nouvelles stratégies mises en œuvre pour soigner ces infections.

Mots-clés

Tratamiento de infecciones micobacterianas

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Resumen
Debido a algunas de las propiedades de las micobacterias y de sus huéspedes, el tratamiento de las infecciones micobacterianas difiere del de otras enfermedades bacterianas. Una de las principales características de las micobacterias radica en la compleja envoltura celular, rica en lipidos, que protege al microorganismo tanto de la respuesta del huésped como de las terapias antimicobacterianas. Esas bacterias son además parásitos intracelulares facultativos, que suelen provocar un tipo de enfermedad de carácter más crónico. Esas propiedades dificultan considerablemente la aplicación de una terapia eficaz. Para que resulten efectivos, los fármacos han de ser capaces de penetrar también en los macrófagos del huésped, y es preferable además que tengan poca toxicidad y resulten eficaces a dosis bajas, haciendo posible de esta manera la aplicación de terapias prolongadas. El autor describe las propiedades generales de la relación huésped-patógeno que se establece en las infecciones micobacterianas, así como las alternativas terapéuticas existentes y los mecanismos de acción de los tratamientos. Con la descripción de nuevos métodos de tratamiento de estas infecciones, el autor ilustra las posibilidades terapéuticas que trae consigo el progreso de la tecnología.

Palabras clave
References


