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From Basic Science to Patient Care

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Chapter 18: Drugs and Drug Interactions

Pedro Almeida da Silva and José A. Aínsa

18.1. Introduction

The history of tuberculosis (TB) changed dramatically after the introduction of anti-mycobacterial agents. Drug treatment is fundamental for controlling TB, promoting the cure of the patients and breaking the chain of transmission when the antituberculosis drug regimen is completely and correctly followed.

Antituberculosis drug treatment started in 1944, when streptomycin (SM) and para-aminosalicylic acid **(**PAS) were discovered. In 1950, the first trial was performed comparing the efficacy of SM and PAS both as monotherapy or combined. The study demonstrated that combined therapy was more effective and resulted in the first multidrug antituberculosis treatment that consisted of a long course of both drugs. In 1952, a third drug, isoniazid (INH), was added to the previous combination, greatly improving the efficacy of treatment, but which still had to be administered for 18-24 months. In 1960, ethambutol (EMB) substituted PAS, and the treatment course was reduced to 18 months. In the ’70s, with the introduction of rifampicin (RIF) into the combination, treatment was shortened to just nine months. Finally, in 1980, pyrazinamide (PZA) was introduced into the antituberculosis treatment, which could be reduced further to only six months.

Two biological features explain why combined drug therapy is more effective at curing TB than monotherapy. One is that treatment of active TB with a single drug results in the selection of drug resistant bacilli and failure to eliminate the disease. The other is that different populations of tubercle bacilli – each of them showing a distinct pattern of susceptibility for antituberculosis drugs – may co-exist in a TB patient (Shamputa 2006).

Soon after the introduction of the first anti-mycobacterial drugs, drug resistant bacilli started to emerge, but the launch of both combination therapy and new and more effective drugs seemed to be enough to control the disease. In fact, it was thought that TB could be eradicated by the end of 20th century. However, TB unexpectedly re-emerged in the ’80s, and in the following years there was an important increase in the incidence of poly-, multiple-, and extensively drug resistant strains. Since 1970, no new drug has been discovered for antituberculosis treatment, which today seems insufficient to confront the disease. Fortunately, research efforts have been accomplished and today there is a wide range of new molecules with promising antituberculosis activity.

In our days, due to the worldwide re-emergence of TB and the increased incidence of multidrug resistant (MDR) and extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis* (Centers for Disease Control and Prevention 2006, see also Chapter 19), new anti-mycobacterial agents (see section 18.6 below), new drug delivery systems (Gelperina 2005), and new treatment regimens are being investigated.

In this chapter, we describe the basic guidelines on TB treatment along with a description of the major antituberculosis drugs (the classical drugs) and their pharmacokinetic properties, toxicity, and interactions with other drugs. Mechanisms of drug resistance in the tuberculous bacillus are also described. In the final part of this chapter we review the main new antimycobacterial drugs that are being developed as candidates to be incorporated in the arsenal of anti-tuberculosis drugs.

18.2. Overview of existing treatment schemes

18.2.1. Rationale

Antituberculosis treatment has two main objectives (Onyebujoh 2005). First, there is a need to rapidly kill those bacilli living extracellularly in lung cavities, which are metabolically active and are dividing continuously; this is required in order to attain the negativization of sputum and therefore to prevent further transmission of the disease. Second, it is necessary to achieve complete sterilization and elimination of those bacilli replicating less actively in acidic and anoxic closed lesions, and to kill semi-dormant bacilli living intracellularly in other host tissues, otherwise these bacilli may persist and will be responsible for subsequent TB relapses. INH is the drug with the highest activity against rapidly dividing bacilli, whereas RIF and PZA have the greatest sterilizing activity against bacteria that are not dividing. These reasons, along with the prevention of drug resistance, support the use of a combination therapy for the treatment of TB.

Drugs for treating TB are usually classified as first- and second-line drugs. Traditionally, there are five first-line drugs: INH, RIF, PZA, EMB, and SM. Second-line drugs include the aminoglycosides kanamycin and amikacin, the polypeptide capreomycin, PAS, cycloserine, the thioamides ethionamide and prothionamide and several fluoroquinolones such as moxifloxacin, levofloxacin and gatifloxacin. Some reports, however, include SM among the second-line drugs, since its use has declined in recent years, due to the high rates of resistance, and also, because other more effective drugs have been incorporated into the anti-tuberculosis treatment. Similarly, new drugs such as the rifamycin derivatives rifapentine and rifabutin can

be considered among the first-line drugs, and in the near future, it is quite likely that some fluoroquinolones could be incorporated into the standard anti-tuberculosis treatment, thus being considered as first-line drugs.

The current short-course treatment for the complete elimination of active and dormant bacilli involves two phases:

- **initial phase**: three or more drugs (usually isoniazid, rifampicin, pyrazinamide and ethambutol or streptomycin) are used for two months, and allow a rapid killing of actively dividing bacteria, resulting in the negativization of sputum
- **continuation phase**: fewer drugs (usually isoniazid and rifampicin) are used for 4 to 7 months, aimed at killing any remaining or dormant bacilli and preventing recurrence

18.2.2. Dosing

There are five first-line drugs: INH, RIF, EMB, PZA, and SM. For standard regimes, first-line drugs should be used at the doses summarized in Table 18-1 (data from Martindale 2004, and Centers for Disease Control and Prevention 2003a).

Table 18-1: Recommended doses for first-line antituberculosis drugs

Drug	Adults or Children[a]	Daily dose (max. dose)	Three times per week (max. dose)	Twice per week (max. dose)
INH[b]	Adults	5 mg/kg (300 mg)	10-15 mg/kg (900 mg)	15 mg/kg (900 mg)
	Children	10-15 mg/kg (300 mg)		20-30 mg/kg (900 mg)
RIF	Adults	10 mg/kg (600 mg)	10 mg/kg[c] (600 mg)	10 mg/kg[c] (600 mg)
	Children	10-20 mg/kg (600 mg)		10-20 mg/kg (600 mg)
PZA[d]	Adults	18.2-26.3 mg/kg (1-2 g)	27.3-39.5 mg/kg (1.5-3 g)	36.4-52.6 mg/kg (2-4 g)
	Children	15-30 mg/kg (2 g)		50 mg/kg (2 g)
EMB[d]	Adults	14.5-21.1 mg/kg (800-1,600 mg)	21.8-31.6 mg/kg (1.2-2.4 g)	36.4-52.6 mg/kg (1.2-2.4 g)
	Children	15-20 mg/kg (1 g)		50 mg/kg (2.5 g)
SM	Adults	15 mg/kg[e] (1 g)		
	Children	20-40 mg/kg (1 g)		20 mg/kg (1 g)

[a]: Patients under 15 years of age.

[b]: INH can be given also once per week, on a 15 mg/kg basis, up to a maximal dose of 900 mg

[c]: For RIF, some manuals also recommend higher doses (10-15 mg/kg) intermittently (two-three days per week) having a maximum of 900 mg (Martindale 2004)

[d]: For PZA and EMB, doses have to be calculated precisely depending on the weight range (for details, see CDC 2003a)

[e]: SM: doses should be reduced to 10 mg/kg in people over 59 years old

When resistance to any of these first-line drugs is found or highly suspected, or when adverse effects to first-line drugs develop during therapy, the treatment should include other drugs known as second-line drugs (details of second-line drugs can be found in section 18.3). The doses and periodicity of second-line drugs and other drugs are given in Table 18-2 (Centers for Disease Control and Prevention 2003a).

Table 18-2: Recommended doses for second-line anti-tuberculosis drugs

Drug	Adults or children[a]	Dose (max. dose)	Days per week
Rifapentine[b]	Adults	10 mg/kg (600 mg)	One
Rifabutin[c]	Adults	5 mg/kg (300 mg)	Two, three or seven
Cycloserine	Adults and children	10-15 mg/kg (1 g)	Seven
Ethionamide	Adults and children	15-20 mg/kg (1 g)	Seven
Amikacin	Adults	15 mg/kg (1 g)	One, two, three or seven
Kanamycin	Children	15-30 mg/kg (1 g)	Two or seven
Capreomycin			
PAS	Adults	8-12 g	Seven
	Children	200-300 mg/kg (10 g)	Seven
Levofloxacin[d]	Adults	500-1,000 mg	Seven
Moxifloxacin[d]	Adults	400 mg	Seven
Gatifloxacin[d]			

[a]: Patients under 15 years of age.

[b]: This drug has not been approved for use in children.

[c]: Doses of rifabutin may need to be adjusted in HIV-positive patients receiving antiretroviral therapy.

[d]: This drug has not been approved for long-term use in children and adolescents.

18.2.3. Treatment regimens

There are many different anti-tuberculosis regimens described in the literature, mostly matching the premises, indications and doses indicated in the sections above (Centers for Disease Control and Prevention 2003a, World Health Organization 2003). Several drug regimens are recommended depending on many factors, such as disease localization and severity, result of sputum smear microscopy, human immunodeficiency virus (HIV) co-infection, prevalence of drug resistance in the setting, availability of drugs, cost of treatment and medical supervision, whether the patient has previously received any anti-tuberculosis drug, the country's budget, health coverage by public health services, and qualifications of health staff at the peripheral level. Then, the selection of a particular drug regimen must be done considering all these factors.

The World Health Organization (WHO) has established four TB diagnostic categories, assuming from a public health perspective that the highest priority of national

TB programs is to identify and cure those patients with sputum smear-positive pulmonary TB, i.e. infectious TB patients (World Health Organization 2003). **Category I** comprises those patients with a high priority for treatment who are new smear-positive patients, new smear-negative pulmonary TB patients with extensive parenchymal involvement, patients with concomitant HIV/acquired immunodeficiency syndrome (AIDS) disease or severe forms of extrapulmonary TB. Patients with a lower priority for treatment are classified as follows: **Category II** (relapse, treatment failure or default), **Category III** (new smear-negative pulmonary TB other than in Category I and less severe forms of extrapulmonary TB) and **Category IV** (chronic sputum-positive TB after re-treatment and proven or suspected MDR-TB). Preferred and optional treatment regimens for each category, as recommended by the WHO, are detailed in Table 18-3 at http://www.tuberculosistextbook.com/pdf/Table 18-3.pdf .

In addition to these guidelines for TB treatment, there are other alternatives. For example, the Center for Disease Control and Prevention (CDC) of the United States (US) also suggests continuation phases consisting of INH and rifapentine once per week for four months for patients in Category I (Centers for Disease Control and Prevention 2003a). This treatment can be used when sputum is negative for acid-fast bacilli (AFB) after the first two months of treatment but should be extended to nine months if the result of the culture at that time point is sill positive. These guidelines apply only to HIV-negative patients as regimens containing rifapentine should not be administered to HIV/AIDS patients.

In general, the duration of the continuation phase must be estimated once the first two months of treatment (initial phase) have been completed. If the patient had cavitations on initial chest radiography and cultures are still positive after two months of treatment, the continuation phase should be extended to 31 weeks (seven months).

When drug resistance develops, patients should be treated with a new combination containing at least three drugs that they had never received before (or that do not show cross-resistance with those to which resistance is suspected). In these conditions, the treatment is longer, more toxic, more expensive and less effective than regimens containing first-line drugs, and should be directly observed.

In children, drug regimens similar to those described above for adults can be given, although EMB is not recommended because of its ocular toxicity. Rifapentine has not been approved for pediatric use.

In case of pregnancy, similar drug regimens can be prescribed, although SM and other second-line aminoglycosides must not be given because they are ototoxic for

the fetus. Also, there has been concern about the use of PZA. Then, a drug regimen of nine months of INH and RIF supplemented with EMB during the first months has been proposed. All antituberculosis drugs are compatible with breast feeding, although babies should be given chemoprophylaxis for at least three months after the mother is considered non-infectious.

Since HIV/AIDS patients have a higher probability of acquiring TB (either pulmonary or extrapulmonary) or other mycobacterial opportunistic infections, particular drug regimens have been designed for treating active TB disease in them (Tuberculosis Coalition for Technical Assistance 2006). Also, the severity of adverse effects of anti-mycobacterial drugs (due to the interactions with anti-retroviral drugs) and mortality is higher among HIV-positive patients. Although, in general, HIV-positive patients respond well to a standard short-course treatment of TB, treatment failure due to malabsorption of antimycobacterial drugs has been reported. The WHO recommends not using SM or thiacetazone in HIV-positive patients in order to prevent the adverse effects of these drugs, often enhanced by anti-retroviral drugs; EMB can be used instead. Rifamycins (rifampicin, rifabutin, etc.) have clinically relevant interactions with some drugs used in the antiretroviral therapy, since they induce the metabolism of anti-retroviral agents such as zidovudine, non-nucleoside reverse transcriptase inhibitors, and HIV protease inhibitors, whose concentrations may fall to sub-therapeutic levels (see section 18.5 below). Then, rifamycin-free regimens have been suggested. They consist of INH, EMB, PZA, and SM, daily for two months, followed by INH, PZA, and SM two or three times weekly for seven months. However, it has also been described that the use of RIF throughout antituberculosis treatment improves outcome in HIV patients.

Chemoprophylaxis of TB is indicated for asymptomatic patients having a positive tuberculin skin test (TST) but not showing active disease (latent TB infection), especially when they are at risk of developing the disease (for example, HIV-positive patients) (Balcells 2006, Centers for Disease Control and Prevention 2003b, Stout 2004). This is aimed at preventing the occurrence of TB. Prophylaxis is most frequently achieved by the administration of INH only, at doses of 300 mg daily for six to nine months (although there is a risk of developing INH resistance). When resistance to INH is suspected, other regimens include RIF, PZA or EMB, can be administered, although there is a greater chance of having adverse effects. In TB prophylaxis, RIF can be given concurrently with INH, reducing the prophylaxis treatment to three months.

It is of prime importance to ensure the patient's adherence to the antituberculosis treatment in order to achieve complete elimination of the bacilli (and hence avoid disease relapse), and also to prevent the emergence of drug resistance. For this

reason, the antituberculosis treatment has to be supervised. Both the patient's adherence and supervision are often difficult to manage when the antituberculosis treatment has to be administered on a daily basis. Alternative treatments based on an intermittent administration of drugs (three times, twice and even once per week) facilitate the patient's adherence and the supervision of treatment. Intermittent treatment is possible because antituberculosis drugs have a marked post-antibiotic effect. After the tuberculous bacillus has been exposed to drugs, there is a lag period (up to several days) during which its growth is interrupted even after the drug concentration has fallen to sub-inhibitory levels. Thus, there is no need to maintain a continuous inhibitory drug concentration to kill the bacilli or prevent growth.

18.2.4. Drug preparations

Most drugs used in antituberculosis treatment – INH, RIF, rifapentine, rifabutin, PZA, EMB, and ethionamide (ETH) – are commercially available as tablets or capsules and can therefore be taken orally. INH is also available as an elixir, in granules for pediatric use, and in aqueous solution for intravenous or intramuscular injection. RIF is available in powder for preparing suspensions for oral administration, and also in aqueous solution for intravenous or intramuscular injection. The exceptions are the aminoglycosides – SM, kanamycin, and amikacin – and capreomycin, which are only available as aqueous solutions for intravenous or intramuscular injection. PAS is usually available as granules for mixing with food; tablets and solutions for intravenous administration can also be found. The fluoroquinolones are available as tablets or as aqueous solutions for intravenous injections.

The three main drugs used in the standard antituberculosis regimen – INH, RIF, and PZA – can also be found in fixed-dose combination preparations (Centers for Disease Control and Prevention 2003a, Panchagnula 2004, World Health Organization 2003). There are several combinations, containing for example, INH and RIF, INH and EMB, INH, RIF and PZA, and INH, RIF, PZA and EMB. When available, the use of combination preparations is recommended. Indeed, by reducing the number of tablets to be taken, they facilitate the patient's adherence to treatment and supervision of therapy. Most importantly, this form of preparation minimizes the possibility of monotherapy and therefore, reduces the risk of drug resistance development.

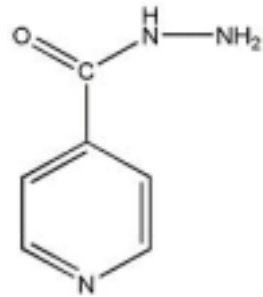
18.3. Drugs: structure, pharmacokinetics and toxicity

In this section, we describe the structure, general properties, pharmacokinetics, and toxicity of the main drugs used in the treatment of TB. More detailed information on drugs can be found in pharmacological books (Martindale 2004), reports on TB treatment (Centers for Disease Control and Prevention 2003a) or highly specialized reports (Douglas 1999, Forget 2006, Launay-Vacher 2005, Nuermberger 2004a, Saukkonen 2006, Zhang 2005).

18.3.1. Isoniazid

Structure and general properties

INH is a pro-drug that requires processing by the bacterial catalase-peroxidase to become active. Once activated, it inhibits the biosynthesis of mycolic acids, which are essential components of the mycobacterial cell wall. This drug is bactericidal against metabolically active bacilli and bacteriostatic against resting bacilli. INH is active against *M. tuberculosis, M. bovis* and *M. kansasii.* Susceptible *M. tuberculosis* strains show minimal inhibitory concentrations (MIC) between 0.02 and 0.2 mg/L.

Isoniazid (isonicotinic acid hydrazide; $C_8H_7N_3O$, MW: 137.1) is one of the most powerful drugs against TB. It is a white crystalline powder freely soluble in water. Solutions can be sterilized by autoclaving	

Pharmacokinetics

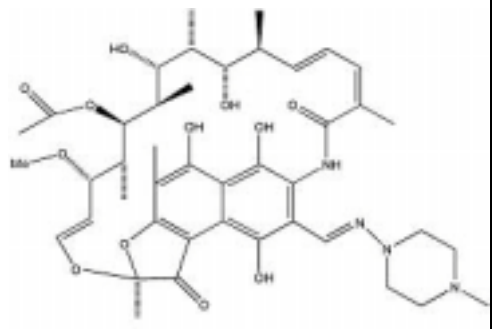
INH is readily absorbed from the gastrointestinal tract (although absorption is reduced by food) or following intramuscular injections. Peak concentrations of 3-8 mg/L appear in blood between 1-2 hours after ingestion of 300 mg of INH. It diffuses into all body tissues, including cerebrospinal fluid. The plasma half-life ranges from 1 to 6 hours. INH is metabolized in the liver and the small intestine: first, an N-acetyltransferase acetylates INH producing acetylisoniazid; this product is hydrolyzed to isonicotinic acid and monoacetylhydrazine, and the latter compound is further acetylated to diacetylhydrazine. None of these INH-derived metabolites have any antituberculosis activity. Within the population, there are two groups of patients, depending on whether INH is acetylated slowly or rapidly, a characteristic that is genetically determined. Plasma INH concentrations are lower in rapid acetylators than in slow acetylators, although this difference does not affect the efficacy of the treatment. INH and its metabolites are excreted in the urine.

Toxicity

INH is well tolerated at recommended doses, although slow acetylators can accumulate higher INH concentrations and then have a higher risk of developing adverse effects. Between 10 % and 20 % of patients may develop transient increases in liver enzymes at the beginning of treatment, and sometimes develop hepatic damage. In these cases, administration of INH should be stopped. Liver function should be monitored before and during treatment, especially in those patients with a history of hepatic or renal dysfunction, in whom doses of INH should be reduced to prevent further damage. Neurological or hematological adverse effects and hypersensitivity reactions occur less frequently. A daily dose of 10 mg of pyridoxine hydrochloride is recommended to reduce neurotoxicity and to treat adverse effects caused by INH.

18.3.2. Rifampicin

Structure and general properties

Rifampicin, often spelled rifampin, (5,6,9,17,19,21-Hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-[*N*-(4-methyl-1-piperazinyl)formimidoyl]-2,7-(epoxypen-tadeca[1,11,13]trienimino)-naphtho[2,1-*b*]furan-1,11(2*H*)-dione21-acetate; $C_{43}H_{58}N_4O_{12}$; MW 822.9) is a red-brown crystalline powder poorly soluble in water; it is dissolved in methyl alcohol and can be stored at room temperature protected from light.	

RIF inhibits gene transcription, by interacting with the beta subunit of the ribonucleic acid (RNA) polymerase enzyme. It is bactericidal against dividing mycobacteria and also has some activity against non-dividing bacilli. *M. tuberculosis* strains are normally susceptible to 0.1-2 mg/L. The introduction of RIF, thus, allowed reduction of the duration of standard antituberculosis treatments from one year to nine months. This was later reduced to six months after incorporation of PZA. RIF is also active against a wide range of microorganisms, including staphylococci, *Neisseria* spp. *Haemophilus influenzae* and *Legionella* spp.

Pharmacokinetics

This drug is readily absorbed from the gastrointestinal tract (food may delay or decrease RIF absorption); within 2 to 4 hours after ingestion of a dose of 600 mg, peak plasma concentrations may reach 7-10 mg/L. It also can be given intravenously. In blood, RIF is bound to plasma proteins, and distributes into body tissues and fluids, including cerebrospinal fluid and breast milk, and crosses the placenta.

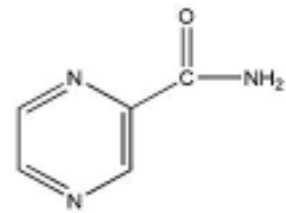
The half-life of RIF ranges from 2 to 5 hours. RIF is metabolized in the liver, and excreted in the bile, feces and urine.

Toxicity

RIF is well tolerated, although adverse effects may arise during intermittent therapy or when restarting an interrupted treatment. Adverse effects include diverse alterations in the gastrointestinal tract, skin, kidney and nervous system. It may also produce thrombocytopenia. RIF will cause a red-orange coloration of body fluids such as urine, tears, saliva, sweat, sputum and feces; it may result in the coloration of soft contact lens. Since it is metabolized in the liver, hepatic functions should be controlled before starting treatment and monitored regularly until the therapy ends. Special care should be taken in patients with pre-existing liver diseases. A moderate increase in alkaline phosphatase can be observed.

18.3.3. Pyrazinamide

Structure and general properties

Pyrazinamide (pyrazinoic acid amide, $C_5H_5N_3O$; MW: 123.1) is a white crystalline powder, soluble in water.	

PZA is a bactericidal drug active only against *M. tuberculosis*, having no in vitro activity against other mycobacteria or any other microorganism. Susceptible strains have MICs of 20 mg/L at pH 5.6. It is active against persisting and non-dividing bacilli, even against those residing intracellularly, being almost inactive at neutral pH. The introduction of PZA into treatment regimens for TB allowed reduction of the duration of such regimens to six months. PZA is a pro-drug that requires conversion into pyrazinoic acid to be effective; this is done by mycobacterial pyrazinamidases.

Pharmacokinetics

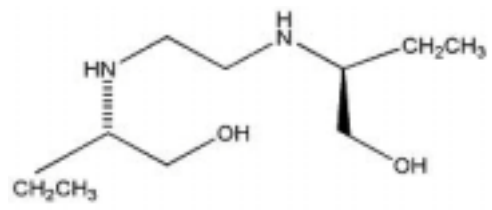
PZA is given orally and is readily absorbed from the gastrointestinal tract. Serum concentrations reach a peak level of about 66 mg/L two hours after administration of a dose of 3 g. It is distributed in all body tissues and fluids, including the cerebrospinal fluid and breast milk. The half-life of PZA is about 9-10 hours. PZA is hydrolyzed in the liver, being converted to pyrazinoic acid, which is further hydroxylated and finally excreted in the urine.

Toxicity

PZA is hepatotoxic in a dose-dependent manner. Following a daily dose of 3 g of PZA, 15 % of patients may develop liver alterations, such as transient increases in liver enzymes, hepatomegaly, splenomegaly and jaundice. Hepatitis has been reported in less than 3 % of cases. It may also produce hyperuricemia, leading to attacks of gout. Therefore, it is contra-indicated in patients with liver damage, and it is advisable to test liver function before and regularly during treatment. It also should not be given to patients having a history of gout or hyperuricemia.

18.3.4. Ethambutol

Structure and general properties

Ethambutol (N,N'-ethylenebis(2-aminobutan-1-ol) dihydrochloride; $C_{10}H_{24}N_2O_2$,2HCl; MW: 277.2) is a white crystalline powder soluble in water and alcohol that must be stored preserved from air.	

This drug is used to treat TB and other opportunistic infections caused by non-tuberculous mycobacteria such as *Mycobacterium kansasii*. The MICs of sensitive *M. tuberculosis* strains range from 0.5 to 8 mg/L.

EMB is only active against dividing mycobacteria, being bacteriostatic. Since EMB affects the biosynthesis of the cell wall, it has been suggested that it contributes towards increasing the susceptibility of *M. tuberculosis* to other drugs.

Pharmacokinetics

EMB is given orally, as it is well absorbed in the gastrointestinal tract (and not affected significantly by food), although a part is excreted in the feces. After absorption, it is distributed in most tissues and diffuses into the cerebrospinal fluid and breast milk; it also crosses the placenta. Following a dose of 25 mg/kg body weight a peak concentration of 5 mg/L in serum is reached after 4 hours. The half-life is about 3 to 4 hours. Only a fraction of EMB is metabolized in the liver; the unchanged drug and its metabolites are excreted in the urine.

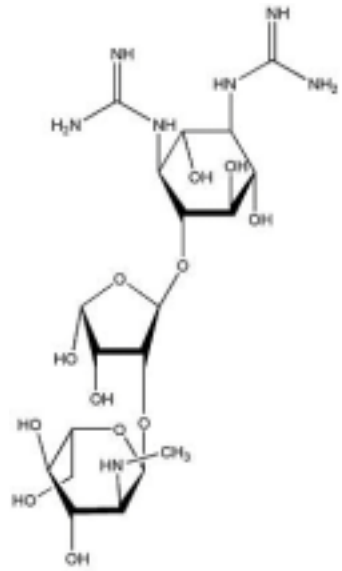
Toxicity

EMB produces retrobulbar neuritis with a reduction in visual acuity, constriction of visual field, central or peripheral scotoma, and green-red color blindness (Fraunfelder 2006). This may affect one or both eyes. The severity of these effects depends on the dose and duration of treatment. Usually, normal vision is recovered a few weeks after the end of the treatment, although in some cases, this recovery may not occur until some months after the completion of treatment. Consequently, EMB is contraindicated in patients with optic neuritis, and should be used with care in patients with visual disorders. Optical examinations are advisable before and during treatment. EMB is not usually given to children under six years of age because of the difficulty in monitoring visual acuity, unless resistance to INH or RIF is highly suspected.

Other adverse effects include a reduction of urate excretion (hence producing gout), gastrointestinal disorders and hypersensitivity skin reactions.

18.3.5. Streptomycin

Structure and general properties

Streptomycin (O-2-deoxy-2-methylamino- α-L-glucopyranosyl-(1-2)-O-5-deoxy- 3-C-formyl-α-L-lyxofuranosyl- (1-4)-N,N-diamidino-D-streptamine; $C_{21}H_{39}N_7O_{12}$; MW: 581.6). It is a white-whitish crystalline powder, highly hygroscopic and soluble in water that must be stored in airtight containers.	

SM, an antibiotic produced by some strains of *Streptomyces griseus*, was the first drug with antituberculosis activity to be discovered. It is mainly used in the treatment of TB (most *M. tuberculosis* strains are susceptible to 1-8 mg/L of streptomycin). It can also be used in the treatment of other bacterial infections such as those produced by *Yersinia pestis*, *Francisella tularensis*, and *Brucella* spp.

Pharmacokinetics

SM, like most aminoglycosides, is poorly absorbed from the gastrointestinal tract, and therefore it must be administered by intramuscular injection. Because of the toxicity of SM (see below) and the introduction of other drugs that can be administered orally for the treatment of TB, the use of SM has decreased, being relegated to the treatment of infections caused by drug-resistant strains. Two hours after an injection of 1 g SM, drug levels in blood may reach up to 50 mg/L, where one third of it circulates bound to plasma proteins. The half-life for SM is about 2.5 hours.

SM and the other aminoglycosides diffuse well into most extracellular fluids, maybe with the exception of the cerebrospinal fluid. They diffuse quite readily into the perilymph of the inner ear, causing ototoxic effects (see below). Aminoglycosides also tend to accumulate in specific body tissues such as the kidneys. Streptomycin does not appear to be metabolized, and is excreted unchanged in the urine.

The concurrence of other diseases may affect the pharmacokinetics of SM and this may become relevant since there is a relatively small difference between the therapeutic and toxic concentrations of aminoglycosides. For example, patients with renal impairment will have increased plasma concentrations of SM, whereas in patients having diseases that cause expanded extracellular fluid volume or increased renal clearance (such as ascites, cirrhosis, heart failure, malnutrition or burns), SM concentrations will be reduced.

Toxicity

Like most aminoglycosides, SM has ototoxic effects affecting vestibular rather than auditory (cochlear) function, which manifest as dizziness and vertigo. It is less nephrotoxic than other aminoglycosides, although it may produce renal failure when administered with other nephrotoxic agents. Regular assessment of both auditory and renal function is recommended. In case of severe adverse effects, SM can be removed by hemodialysis. Paresthesia, neurological symptoms such as peripheral neuropathies, optic neuritis and scotoma, and hypersensitivity skin reactions have also been observed after SM injections.

18.3.6. Other drugs against tuberculosis

Drugs in this group are interesting for one or more of the following features:

- widely used in the past but in our days its use has been relegated by the incorporation of more effective and/or less toxic drugs
- used when resistance to first-line antituberculosis drugs is suspected or confirmed, and are usually denominated second-line drugs
- used when severe adverse effects to other antituberculosis drugs develop
- have been developed recently and, because of their usefulness for the treatment of TB, are potential first-line drugs that could be incorporated soon into standard (and maybe shorter) antituberculosis regimens
- allow intermittent doses, hence facilitating patient’s adherence to antituberculosis treatment

Para-aminosalicylic acid

This compound and its salts are active only against *M. tuberculosis*, which can be inhibited by 1 mg/L of this drug. It is bacteriostatic. PAS can be given orally, in a daily dose of 10-12 g divided into two or three doses. It is well absorbed in the gastrointestinal tract and distributes well throughout the body, although it is poorly distributed in the cerebrospinal fluid. It is metabolized in the intestine and in the liver, and it is excreted mainly in the urine. PAS may produce gastrointestinal side-effects such as nausea, vomiting, diarrhea, and hypersensitivity reactions, and should be administered with care in patients with liver or renal impairment. PAS can be used safely during pregnancy but is not recommended because of the gastrointestinal intolerance. The use of PAS has largely decreased since the introduction of RMP and EMB; however, due to its low cost, it is still in use in low-resource countries.

Capreomycin

This polypeptide is bacteriostatic against several mycobacteria including *M. tuberculosis*; susceptible strains are inhibited by 10 mg/L of capreomycin. Doses, usually of 1 g, must be administered by intramuscular or intravenous injection. Capreomycin is excreted in the urine. It must be given with care to patients with renal, hepatic or auditory dysfunction. Commonly, capreomycin affects the frequency of urination or the amount of urine, increases thirst and may produce loss of appetite, nausea and vomiting. Due to its toxic effects, it must not be given in combination with aminoglycosides such as kanamycin or streptomycin.

Cycloserine

This is a broad-spectrum antibiotic that inhibits many microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Nocardia* spp., *Chlamydia*, and *M. tuberculosis*. Due to its high toxicity, it is only used against bacilli resistant to the main antituberculosis drugs. Doses of 500 mg are given orally twice a day. It is fairly well absorbed in the gastrointestinal tract, being distributed to most tissues and fluids, including cerebrospinal fluid. Cycloserine is metabolized and excreted in the urine. It should be given with care to patients with renal impairment. It may produce diverse adverse reactions involving the central nervous system, from mild headache or restlessness to severe psychosis and seizures, and is therefore contraindicated in patients with epilepsy, depression or anxiety. Hypersensitivity skin reactions have also been described.

Aminoglycosides

Amikacin and kanamycin are active against a range of bacteria including *M. tuberculosis*. Amikacin is also active against atypical mycobacteria that cause opportunistic infections, such as those of the *Mycobacterium avium* complex. Both are considered as second-line antituberculosis drugs; other safer drugs are preferred for the treatment of TB. These antibiotics are often combined with EMB, ciprofloxacin, and macrolides. Like SM (see above) amikacin and kanamycin must be given by intramuscular injections, usually in doses of 0.5-1 g. They are distributed in body tissues and fluids, and cross the placenta but do not reach the cerebrospinal fluid. Like most aminoglycosides, amikacin and kanamycin affect auditory function and must be given with care to patients with auditory dysfunctions. They are also nephrotoxic, producing renal impairment in approximately 8 % of patients. Kanamycin may also produce some gastrointestinal effects, such as nausea, vomiting, and stomatitis, especially when taken by mouth. Both aminoglycosides are excreted unchanged in the urine.

Thioamides

There are two main drugs from the thioamide (or thionamide) family that can be used for the treatment of TB: ETH and prothionamide.

ETH is a structural analogue of INH and in fact some cross-resistance has been observed between both drugs. ETH is active against *M. tuberculosis, M. leprae, M. kansasii*, and some strains of the *M. avium* complex. Susceptible *M. tuberculosis* strains are inhibited by 0.6-2.5 mg/L of ETH. For the treatment of TB, doses of 15-20 mg/kg of body weight are given orally, up to a maximum of 1 g daily. It is well absorbed from the gastrointestinal tract, and diffuses into all body tissues and fluids, including cerebrospinal fluid. Its half-life is 2 hours. ETH is metabolized in the liver and excreted in the urine. Thus, it should not be given to patients with liver dysfunction. Adverse effects associated with ETH administration include dose-related gastrointestinal disorders (such as anorexia, excessive salivation, nausea, vomiting, metallic taste, abdominal pain, and diarrhea), diverse mental disturbances (such as depression, anxiety, psychosis, dizziness, drowsiness, and headache) and hypersensitivity skin reactions have also been described.

Prothionamide is very similar to ETH; complete cross-resistance between these two drugs usually occurs. It can be used orally, at doses similar to those of ETH. It is well absorbed from the gastrointestinal tract, and distributes to all body tissues and fluids, including cerebrospinal fluid. Prothionamide is metabolized in the liver and excreted in the urine.

Fluoroquinolones

Among the fluoroquinolones, there are drugs with several degrees of activity against *M. tuberculosis* (Ginsburg 2003). Whereas norfloxacin has no activity against mycobacteria, ciprofloxacin and ofloxacin have been used for the treatment of TB, especially when caused by drug resistant strains, and also in the treatment of opportunistic mycobacterial infections. Other fluoroquinolones such as sparfloxacin, gatifloxacin, and moxifloxacin are even more active than ciprofloxacin for the treatment of TB, being comparable to INH.

Fluoroquinolones are well absorbed from the gastrointestinal tract (presence of food reduces absorption), and peak plasma concentrations are obtained rapidly, usually after 1-2 hours, where they are partially bound to plasma proteins. Half-life is variable, ranging from 4 hours in the case of ciprofloxacin to 10-13 hours in the case of moxifloxacin. They distribute well into all body tissues, and are finally, eliminated in the urine. Fluoroquinolones are generally well tolerated. Adverse effects include disorders of the gastrointestinal tract, nervous system, and skin. They should not be given to patients having central nervous system disorders such as epilepsy. The use of fluoroquinolones is not recommended in children or during pregnancy. Interactions with other drugs are infrequent.

Rifamycins

The rifamycin family of drugs includes RIF, one of the most potent first-line antituberculosis drugs (see above). Other members of this family include rifabutin and rifapentine, which share their mode of action and spectrum of antibacterial activity with RIF. A high degree of cross-resistance among rifamycins has been found. Rifapentine and rifabutin have, however, some distinct properties in comparison to RIF, which makes them very useful in certain situations.

The MICs of rifabutin for *M. tuberculosis* susceptible strains are usually eight times lower than those for RIF. Rifabutin can be used for the treatment of TB at doses of 150-450 mg daily, combined with other drugs to avoid drug resistance. It is also frequently used for the prophylaxis of *M. avium* infections in immunocompromised patients and for the treatment of other opportunistic infections caused by mycobacteria.

In contrast to RIF, rifabutin is poorly absorbed in the gastrointestinal tract; once it gets into the blood, most of it is bound to plasma proteins, and distributes widely into the body. Rifabutin is metabolized in the liver where it induces microsomal enzymes, although to a lesser extent than RIF. Rifabutin is excreted in the urine.

Rifabutin produces a syndrome of polyarthralgia-arthritis at doses over 1 g daily. Uveitis has been reported in patients also receiving macrolides or azole antifungals. Rifabutin reduces the plasma concentration of several antiretroviral drugs, such as zidovudine. Despite this, rifabutin (at reduced doses) has been recommended in place of RIF in the treatment of TB in HIV/AIDS patients, in order to avoid major interactions of RIF (see below) and the antiretroviral drugs.

Rifapentine is considered a long-acting rifamycin, since it can be given orally at doses of 600 mg twice weekly or even once weekly during the initial phase in the treatment of TB (Temple 1999). It is well absorbed from the gastrointestinal tract. Rifapentine and RIF show cross-resistance. Adverse effects of rifapentine are similar to those of RIF, except for a higher incidence of hyperuricemia. This drug has not been approved for use in children, since the safety and efficacy of this drug has not yet been established for this age group. Also, rifapentine is not recommended for HIV/AIDS patients because of their risk of developing rifamycin resistance.

Thiacetazone

This drug, also spelled thioacetazone, is bacteriostatic against *M. tuberculosis*, with susceptible strains being inhibited by 1 mg/L. Cross-resistance with ethionamide and prothionamide can occur. It may be used in anti-tuberculosis regimens, although these may not be as effective as the standard short-course therapy. It is well absorbed in the gastrointestinal tract and peak concentrations of 1-2 mg/L are obtained four hours after administration of a 150 mg dose. It is excreted in the urine. Thiacetazone produces diverse adverse effects such as gastrointestinal disorders, and hypersensitivity reactions (including skin rashes) that may be more frequent in HIV/AIDS patients. Other frequent adverse effects include conjunctivitis, vertigo, toxic epidermal necrolysis, exfoliative dermatitis, hemolytic anemia, and hepatotoxicity with jaundice. It should not be given to patients with liver impairment, or to HIV/AIDS patients because of the risk of increased adverse reactions. Some low-income countries still use thiacetazone because of its low cost.

18.4. Drug resistance mechanisms

18.4.1. Natural drug resistance

The natural drug resistance of *M. tuberculosis* is an important obstacle for the treatment and control of TB. This resistance has traditionally been attributed to the unusual multi-layer cell envelope and active multidrug efflux pumps (De Rossi 2006, Jarlier 1994). Recent insights into mechanisms that neutralize the toxicity of

antibiotics in the cytoplasm have revealed other systems that function in synergy with the permeability barrier and efflux systems to provide natural resistance. Drugs inhibiting these intrinsic systems would enable many antibiotics, which are already available but have not been used for TB, to gain a new potential use against *M. tuberculosis* (Lomovskaya 2006, Nguyen 2006).

18.4.2. Acquired drug resistance

Knowledge of the molecular basis of drug resistance in *M. tuberculosis* increased with the sequencing of the genome and the development of molecular tools (Aínsa 2001, Cole 1998). In other bacterial species, acquired drug resistance is mediated by plasmids or transposons, but in contrast, *M. tuberculosis* acquired drug resistance is caused by mutations in chromosomal genes (Heym 1994). So far, no single pleiotropic mutation has been found in *M. tuberculosis* to cause a MDR phenotype. The MDR phenotype is caused by sequential accumulation of mutations in different genes involved in resistance to individual drugs, due to inappropriate treatment or poor adherence to treatment (Zhang 2000). However, it is important to observe that some resistant strains do not present these classic mutations, suggesting the possibility of the existence of other mechanisms such as efflux pumps and alterations in the permeability of the cell wall.

Isoniazid and ethionamide

INH was synthesized in 1912 by the Czech chemists Hans Meyer and Josef Mally, but it was not until 1952 that it was introduced as an antituberculosis agent. The first indication of the mechanism of action of INH was obtained from the observation that as soon as the treatment with INH began, the acid-fast property of the tubercle bacillus was quickly lost. In 1970, it was demonstrated that INH inhibits mycolic acids synthesis, which explained the microscopic observation of the loss of the acid-fastness (Blanchard 1996).

INH has a simple structure, containing a pyridine ring and a hydrazide group and both molecules are essential for its high activity against *M. tuberculosis*. Despite its sinple structure, the mode of action of INH is very complex (Bernstein 1952).

An important aspect to underline is that INH is a prodrug; its antibiotic action depends on the bacterial activation by the catalase-peroxidase enzyme (KatG) (Zhang 1992) to generate reactive radicals, which attack multiple targets in *M. tuberculosis* (Zhang 2000).

The mechanisms of action of INH and ETH are similar but their activation mechanisms are different, so that strains resistant to INH due to mutations in *kat*G are still susceptible to ETH, indicating that there must be another enzyme responsible for the activation of ETH (Blanchard 1996).

The main target of INH is the pathway synthesizing cell wall mycolic acids (Takayama 1972). Furthermore, at least two enzymes, InhA (enoyl acyl carrier protein reductase) (Banerjee 1994) and KasA (beta-ketoacyl ACP synthase) (Mdluli 1998) have been identified as targets for INH.

Resistance to INH is mostly associated with mutations or deletions in *katG*; other mutations related with INH resistance occur in the coding region of *inhA* gene (or its promoter) and *kas*A. Furthermore, mutations in several other genes have been reported to be associated with INH resistance, but occur less frequently, and their association with INH resistance is less clear (Sreevatsan 1997; Ramaswamy 2003).

The relationship between the overexpression of *ahp*C and INH resistance has been investigated (Rattan 1998), however, it was demonstrated that the increase in the expression of *ahp*C in INH-resistant strains is aimed at compensating the loss or the decrease in catalase activity produced by the alteration of the *kat*G gene; thus, this increased expression of *ahpC* on its own would not be related with INH resistance (Sherman 1999). Other possible resistance mechanisms are being investigated in *M. smegmatis*, which is 300 times more resistant to INH than *M. tuberculosis*, indicating that efflux pumps could be another possible mechanism of INH resistance (Choudhuri 1999, Colangeli 2005).

Rifampicin

RIF, a lipophilic ansamycin, was introduced in 1972 in the treatment of TB. Due to its efficient antimicrobial action, it is considered, together with INH, to be the basis of the short-course treatment regimen (Rattan 1998).

RIF associates with the beta-subunit of the ribonucleic acid (RNA) polymerase, inhibiting the elongation of the messenger RNA (mRNA) (Blanchard 1996). RNA polymerase is an oligomer consisting of a catalytically competent core enzyme formed by four subunits (two alpha subunits, and beta and beta-prime subunits) in association with another subunit, sigma, which is able to specifically initiate transcription (Zhang 2000).

As in *Escherichia coli*, almost all clinical isolates of *M. tuberculosis* resistant to RIF show mutations in *rpoB*, the gene that encodes the beta-subunit of the RNA polymerase, resulting in conformational changes that determine the low affinity of this subunit for RIF and consequently, resistance to the drug (Jin 1988, Williams

1994). Mutations conferring resistance to RIF are clustered in three short regions in the central region of the beta-subunit gene: cluster I (amino acids 512 to 534), cluster II (amino acids 563 to 574) and cluster III (amino acid 687) (Zhang 2000).

Although mutations in *rpoB* usually result in high-level resistance and show cross resistance to other rifamycins, mutations in codons 511, 516, 518, 522, 529, and 533 have been associated with low-level resistance to RIF and/or susceptibility to rifabutin and the new rifamycin KRM1648 (Bodmer 1995, Cavusoglu 2004, Moghazeh 1996, Yang 1998, Williams 1998).

Pyrazinamide

PZA is structurally similar to nicotinamide, and is converted into the acid form (pyrazinoic acid) by the bacterial pyrazinamidase enzyme (PZase) (Konno 1967). PZA is active against bacilli in a semi-dormant state. Its introduction into the primary treatment of TB allowed the reduction of the treatment from nine to six months. This property has been attributed to its ability to inhibit semi-dormant bacilli residing in acidic environments (Mitchison 1985).

The antimicrobial action of PZA is highly specific for *M. tuberculosis*, with little or no activity against other mycobacteria, including *M. bovis*. The reason for the specific activity of PZA against *M. tuberculosis* is because this drug needs to be activated by the PZase enzyme, which is encoded by the *pncA* gene. This gene is altered in many species of mycobacteria, which are resistant to PZA because they lack an efficient PZase. In *M. bovis*, for example, the substitution of the His residue in position 57 for Asp produces a non-effective PZAse (Konno 1967).

In most cases, resistance to PZA is associated with mutations in *pncA*. PZA-resistant strains have shown a wide range of alterations in the 630 bp of the open reading frame or in the 82 bp of the promoter region (Scorpio 1996).

Some PZA-resistant strains do not present any alterations in the coding region or the promoter of the *pncA* gene. For these strains, it has been postulated that PZA resistance could be due to mutations in an unknown *pncA* regulatory gene (Cheng 2000).

Ethambutol

EMB is a synthetic compound used as first-line drug for anti-tuberculosis therapy in combination with other drugs, as recommended by the WHO. It has been demonstrated that EMB acts on enzymes involved in the biosynthesis of arabinogalactan (Takayama 1989), inhibiting the polymerization of cell wall arabinan of arabinogalactan and of lipoarabinomannan (Mikusova 1995). In *M. tuberculosis*, the

emb operon has three contiguous genes: *embC, embA*, and *embB*, which encode mycobacterial arabinosyl transferases (Telenti 1997). These enzymes have been considered the drug targets for EMB, since substitutions of codon 306 in the *M. tuberculosis embB* gene have been shown to be the most frequent and predictive mutations for EMB resistance (Srivastava 2006). For strains with the Met306Leu or Met306Val replacements, EMB MICs were generally higher (40 mg/L) than those for organisms with Met306Ile substitutions (20 mg/L). In *M. tuberculosis*, mutations in genes other than *embB* have been associated with EMB resistance. Often these mutations affect a putative regulatory sequence in the *embC–embA* intergenic region (Ramaswamy 2000).

Streptomycin

SM is an aminocyclitol glycoside antibiotic that was the first antibiotic used for the treatment of TB. SM inhibits the initiation of mRNA translation affecting translation fidelity (Moazed 1987). Mutations associated with SM resistance in *M. tuberculosis* have been identified in the 16S ribosomal RNA (rRNA) gene (*rrs*) and *rpsL* gene encoding ribosomal protein S12 (Finken 1993). The majority of point mutations producing SM resistance occur in *rpsL* and the most common mutation is an AAG->AGG change in codon 43, which results in a Lys->Arg substitution; less frequently, an AAG->ACG (Lys->Thr) substitution is observed (Böttger 1994, Musser, 1995). The second mechanism of SM resistance in *M. tuberculosis* is mutation in *rrs*. Mutations in *rpsL* and *rrs* have been identified in 50 and 20 % of SM-resistant clinical isolates, respectively, resulting in high or intermediate levels of SM resistance respectively. There are some clinical isolates that show low level SM resistance in which no mutation in *rpsL* or *rrs* has been found (Zhang 2000). It has been hypothesized that changes in the cytoplasm concentration of SM due to the action of efflux pumps could be the molecular basis of SM resistance in these strains (Aínsa 1998, Meier 1996, Silva 2001).

Fluoroquinolones

The main targets of the quinolones are the desoxyribonucleic acid (DNA) gyrase, a type-II DNA topoisomerase composed of two A and two B subunits encoded by genes *gyrA* and *gyrB*, respectively (Takiff 1994), and DNA topoisomerase IV (Drlica 2003). High-level resistance to fluoroquinolones in laboratory strains of *M. tuberculosis* and *M. smegmatis* (Takiff 1994) is known to result from amino acid substitutions in the putative fluoroquinolone binding region of the *M. tuberculosis gyrA* or *gyrB* genes (Aubry 2004, Cambau 1994). This is the only type II topoisomerase encoded in the *M. tuberculosis* genome (Cole 1998) and thus, is the unique target for fluoroquinolones in this organism (Aubry 2004).

Fitness and Drug Resistance

The relation between drug resistance and fitness cost has led to the assumption that removal of antibiotic selective pressure would favor the elimination of resistant bacteria, because mutations conferring drug resistance usually affect replication and this is a disadvantage when resistant bacteria have to compete with sensitive bacteria in the absence of antibiotic (Andersson 1999). In fact, antibiotic resistance, caused by target alteration or by other mechanisms, can confer a reduction in fitness expressed as reduced growth, virulence or transmission (Andersson 2006). However, this cost can be compensated, usually without loss of resistance, by second-site mutations during the evolution of the resistant bacteria (Bjorkman 2000). The effects of resistance mutations on the fitness of *M. tuberculosis* could be important in epidemiological predictions of the spread of MDR strains (Cohen 2003).

There are only limited data available on the effect of different drug resistance conferring mutations on the relative fitness of *M. tuberculosis* (Billington 1999, Bottger 1998, Gagneux 2006, Mariam 2004, Pym 2002). The main limitations in some of these studies are the use of *in vitro* models or non-isogenic strains. Host and environmental factors, as well as strain genetic diversity can also influence the transmission dynamics of drug-resistant bacteria, while virulence of strains may reflect other genomic differences uncoupled from drug resistance.

18.5. Drug interactions

In general, when two or more drugs are administered simultaneously to a patient, there is a possibility that the drugs involved may interact between them. This interaction may result in changes (increase or decrease) of the effective concentration of one or more of the drugs involved, which most can usually be solved by adjusting the doses of the affected drug. The interaction may also produce an enhancement in adverse effects produced by any of the drugs, which is frequently solved by using alternative drugs that are not affected by the interaction. Since the antituberculosis treatment itself consists of the administration of two or more drugs, and in some occasions it is given simultaneously with other drug regimes (i.e. the antiretroviral treatment) it is very important to consider those drug interactions affecting the TB drugs.

Few drugs interact to alter the concentration of the antituberculosis drugs (Centers for Disease Control and Prevention 2003a, Martindale 2004, Yew 2002). More frequently, antituberculosis drugs affect the other drugs. Most of the clinically relevant interactions involve the rifamycin drugs (RIF, rifapentine and rifabutin).

Other interactions affecting first-line antituberculosis drugs and the fluoroquinolones will also be described in this section.

18.5.1. Rifamycins

The rifamycins are metabolized mainly in the liver, and to a lesser extent in the intestine wall, where they induce several pathways involving isoenzymes of the cytochrome P450 system, such as the isoenzyme CYP3A4 (Yew 2002). The extent of the induction of the isoenzyme CYP3A4 depends on the particular rifamycin drug that is being used, and so, RIF is the most potent inducer, whereas rifapentine is a moderate inducer and rifabutin is the least potent inducer of the isoenzyme CYP3A4. Rifabutin, but not RIF or rifapentine, is also a substrate of CYP3A4. Then, other drugs that share or interact with the cytochrome P450 system may have significant levels of interaction with the rifamycins.

Drugs affecting the rifamycins

Ritonavir, a protease inhibitor that is combined with inhibitors of reverse transcriptase during anti-HIV therapy, is a potent inhibitor of the isoenzyme CYP3A4, which is the isoenzyme that metabolizes rifabutin. As a consequence, rifabutin levels may increase up to four-fold, and other rifabutin-derived metabolites may also reach higher levels. This produces a higher probability of having leucopoenia and other adverse effects. RIF can be used instead of rifabutin in order to avoid this interaction.

Efavirenz, another antiretroviral drug, is an inducer of the CYP3A4. Its administration may result in a decrease in the concentration of rifabutin to one third of its normal serum concentrations.

Clofazimine, a drug used in the treatment of leprosy, may reduce the absorption of RIF.

Drugs affected by the rifamycins

Since rifamycins induce microsomal liver enzymes, they accelerate the metabolism of some other drugs reducing their half-lives and their concentrations, sometimes to sub-therapeutic levels. This problem can be solved easily by increasing the dosage of the drugs affected, which have to return to normal doses two weeks after completion of the rifamycin treatment. One exception to this general rule can be the case of oral contraceptives in women, and other contraceptive methods should be recommended.

Maybe, the most important family of drugs affected by the rifamycins is the antiretroviral agents, both the protease inhibitors and the non-nucleoside reverse transcriptase inhibitors.

RIF should not be administered simultaneously with anti-HIV drugs such as zidovudine, non-nucleoside reverse transcriptase inhibitors, and HIV protease inhibitors, since it may induce the metabolism of these drugs in the liver. Rifabutin can be used instead of RIF in some situations. The nucleoside reverse transcriptase inhibitors, which are not metabolized by CYP3A4, can be co-administered with rifamycins.

Other drugs, whose concentrations can be decreased by the use of rifamycins include atovaquone, azathioprine, chloramphenicol, cyclosporine, cimetidine, clofibrate, corticosteroids, coumarin anticoagulants, dapsone, diazepam and other benzodiazepines, doxycycline, fluconazole, haloperidol, hexobarbital, itraconazole, ketoconazole, lamotrigine, methadone, ondansetron, oral hypoglycemics, phenytoin, quinine, rofecoxib, statins, sulphasalazine, tacrolimus, the bronchodilator theophylline, thyroid hormones, and several cardiovascular drugs including beta blockers, digitalis alkaloids and antiarrhythmics such as disopyramide, lorcainide, mexiletine, propafenone, quinidine, tocainide, and verapamil and other calcium-channel blockers.

18.5.2. Isoniazid

Drugs affecting isoniazid

Chronic alcoholism may increase liver metabolism of INH. Aluminum-containing antacids reduce the absorption of INH. Food such as cheese and fish, and also red wine may produce INH-associated adverse effects.

Drugs affected by isoniazid

INH is a potent inhibitor of several cytochrome P450 isoenzymes, and then, it interferes with and inhibits the hepatic metabolism of a large number of drugs (such as, RIF), thus increasing their half-life and therefore their potential toxicity. The main drugs interacting with INH include anti-epileptics such as carbamazepine, ethosuximide and phenytoin, benzodiazepines, and chlorzoxazone.

Combination of isoniazid and rifamycins

In the standard anti-tuberculosis regimes, RIF is administered simultaneously with INH during the complete treatment (initial and continuation phases). Since both drugs are metabolized in the liver, the incidence of hepatotoxicity can be increased

and liver function should be monitored regularly. The risk of hepatotoxicity can also increase when other potentially hepatotoxic drugs are taken.

There is an important number of drugs for which both INH and RIF interact producing opposite effects: INH may increase drug concentrations whereas RIF decreases such concentrations. When both drugs are administered simultaneously, the effect of RIF is more important than that of INH, resulting in a decrease in the concentration of the drugs affected.

18.5.3. Pyrazinamide

Probenecid, a drug used for the treatment of gout, may block the excretion of PZA and co-administration of both drugs also affects excretion of urate. In some cases, patients receiving zidovudine as anti-HIV treatment may have diminished levels of PZA.

18.5.4. Ethambutol

Aluminum hydroxide-containing antacids may reduce the absorption of EMB up to a 20 %. These compounds should be taken at least two hours after the ingestion of EMB to avoid interaction.

18.5.5. Streptomycin

The administration of SM with other nephrotoxic drugs, including other aminoglycosides, vancomycin, and some of the cephalosporins, or potentially ototoxic drugs such as ethacrynic acid or frusemide should be avoided since this could increase the risk of toxicity.

18.5.6. Fluoroquinolones

Several drugs (such as those containing divalent cations, including antacids or vitamin supplements) decrease the absorption of fluoroquinolones (Ginsburg 2003). Taking these medications at least two hours after the dose of fluoroquinolones circumvents this problem.

Some fluoroquinolones can inhibit the metabolism of other drugs, such as the bronchodilator theophylline, therefore enhancing its toxic effects. The most recently developed fluoroquinolones (moxifloxacin, gatifloxacin, etc) lack this effect.

18.6. New drugs for tuberculosis

In the last 40, years no new specific drug, with particular activity against *M. tuberculosis,* has been developed or introduced into the treatment of TB. The available treatment establishes a multidrug regime lasting a minimum of six months, although there is no guarantee that the complete sterilization of the infection will be obtained. Furthermore, the increase in TB cases caused by MDR and XDR strains, and co-infection with HIV have pointed out the urgent need to develop new drugs to treat TB. Research for developing new TB drugs is being conducted using several strategies in different organizations around the world, both in academic institutions and in industrial companies, both financed by private or governmental funds, aimed at researching drugs of either synthetic or natural sources (see World Health Organization at: http://www.who.int/tb/en; TB Alliance at: http://new.tballiance.org/home/home-live.php). An ideal new TB drug should shorten the treatment, kill the persistent bacilli, and be active against resistant strains. Furthermore, the new drug should be specific for *M. tuberculosis*, compatible with existing TB drugs and non-inducer of P-450 enzymes.

In this section, we present some of the main candidates that could be introduced to the therapeutic arsenal of drugs against TB in the near future.

18.6.1. Analogues and derivatives of antituberculosis drugs

The development of new drugs against TB derived from already-known molecules, which have been used in the therapy of TB throughout the years and whose efficacy and safety have been proven, is an attractive strategy from the economic, pharmaceutical and clinical points of view. However, putative cross-resistance with parental molecules could be a negative point. Nevertheless, several analogues and derivatives of the main antituberculosis drugs are being assessed and some preliminary results are promising.

Ethambutol analogues

EMB is one of the main drugs used in the treatment of TB, and in most countries it has replaced SM and thiacetazone. The structure of EMB is favorable to the preparation of analogues by combinatorial chemistry techniques. Some EMB analogues. such as NIH 241 and SQ109 (Figure 18-1), have an efficacy comparable or even better than that of EMB (Protopopova 2005). *In vitro* studies showed that SQ109 interacts synergistically with INH and RIF, and in experimental animal models,

treatments containing SQ109 were 25 % shorter than standard cure of the disease. SQ109 has a narrow spectrum, being active against *M. tuberculosis* and *M. bovis* BCG and less active against *M. smegmatis* and *M. avium*. SQ109 is in Phase I of clinical trials and it could replace one or more of the current first-line anti-tuberculosis drugs, simplify therapy, and shorten the treatment regimen (Jia 2005; Chen 2006).

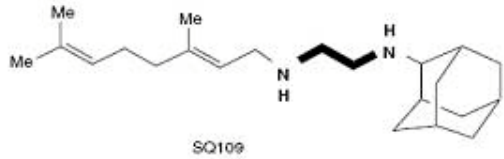


Figure 18-1: SQ109 structure

Isoniazid analogues

Various analogues and derivatives of INH continue to be synthesized. These compounds are likely to be ineffective against INH-resistant strains of TB because of close structural similarities with INH (Hudson 2003). However, since INH is a very important drug of the therapeutic arsenal against TB, efforts are being made to find new INH derivatives with more activity, less toxicity, and fewer side-effects.

Recently, the INH molecule was incorporated into a pyrazoline nucleus, showing activity against strains of *M. tuberculosis*, both susceptible and resistant to INH. Interestingly, other compounds with halogen-substituted phenyl groups showed even more activity (Shaharyar 2006).

In another study, a hydrophobic derivative of INH, 1-isonicotinyl-2-nonanoyl hydrazine, showed enhanced antimycobacterial activity against *M. tuberculosis* H37Rv. It is possible that attachment of chemical groups that help penetration of INH would make *M. tuberculosis* strains more susceptible to this drug (Maccari 2005).

Rifamycin derivatives

RIF is an important drug for the treatment of TB, and its introduction into anti-tuberculosis therapy strongly improved the control of the disease. Some rifamycin derivatives have been developed. Rifabutin shows stronger activity and is used when TB patients are also being treated for HIV infection. This is because rifabutin induces the cytochrome P-450 CYP3A oxidative enzymes at lower levels than other rifamycins (Burman 2001). Rifalazil (KRM1648 or benzoxazinorifamycin)

(Figure 18-2), a new semisynthetic rifamycin with a long half-life, is more active than RIF and rifabutin against *M. tuberculosis* both *in vitro* and *in vivo* in mice. High-level RIF-resistant strains (MIC > 32 mg/L) display cross-resistance to all rifamycins; however, low-level resistant strains (MIC < 32 mg/L) are still susceptible to the new rifamycin derivatives (Zhang 2005).

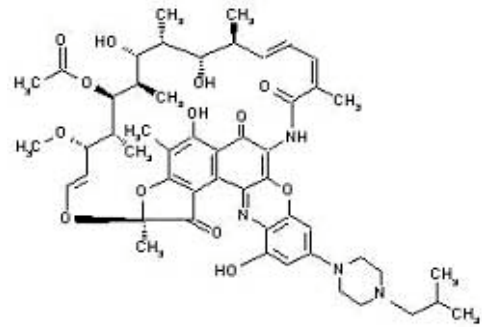
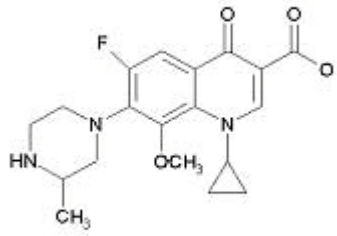


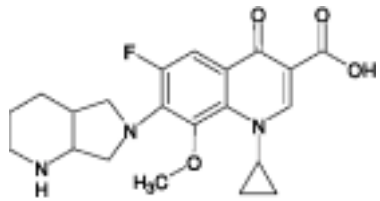
Figure 18-2 Rifalazil structure

Quinolones

Fluoroquinolones have been used sporadically since the late ’80s, primarily for the treatment of TB caused by resistant organisms or because of intolerance to first-line anti-tuberculosis drugs. Two new molecules developed more recently, moxifloxacin and gatifloxacin (Figure 18-3), with longer half-lives, are believed to have the highest *in vitro* activity against *M. tuberculosis*, followed by levofloxacin and ofloxacin (Nahid 2006). Moxifloxacin appeared to kill a subpopulation of tubercle bacilli not killed by RIF (Hu 2003). During Phase II trials, it was found that when gatifloxacin was used instead of ethambutol, the standard six-month regime was shortened to four months (detailed information is available on the internet at the World Health Organization-TDR website: http://www.who.int/tdr/). A recent study showed that moxifloxacin in combination with RIF and PZA was more effective than the classical combination of INH, RIF, and PZA (Nuermberger 2004b). The reason for this could be that moxifloxacin has activity on a subpopulation of microorganisms that is not affected by other drugs, or it could be due to the absence of the antagonism that occurs between INH and PZA (Grosset 1992; Hu 2003). Recently, it was reported that gatifloxacin may cause both hypoglycemia and hyperglycemia in both diabetic and non-diabetic patients (Zvonar 2006; Yamada, 2006), which is a serious obstacle for its use in clinical practice.



Gatifloxacin



Moxifloxacin

Figure 18-3: Structures of gatifloxacin and moxifloxacin

18.6.2. New molecules in clinical trials

DARQ

Diarylquinolines (DARQs) (Figure 18-4) are structurally different from both fluoroquinolones and other quinoline classes. The DARQ R207910 is a promising new drug against TB, because it is bactericidal against both the drug-susceptible and drug-resistant strains of *M. tuberculosis*. Low MICs were also found for other mycobacterial species, including *M. bovis*, *M. kansasii* and *M. ulcerans*, as well as species naturally resistant to many other anti tuberculosis agents that are involved in opportunistic infections, for example, *M. avium* complex, *M. abscessus*, *M. fortuitum*, and *M. marinum.* The activity of R207910 seems to be specific for myco-

bacteria, having much higher MICs for *Corynebacterium, Helicobacter pylori, Nocardia, Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Haemophilus influenzae.* Molecular studies identified the C subunit of ATP synthase as a target of the R207910. Inhibition of ATP synthase function may lead to ATP depletion and imbalance in pH homeostasis, both contributing to decreased bacterial survival. Resistant strains of *M. tuberculosis* and *M. smegmatis* showed mutations in the *atpE* gene that encodes AtpE, a part of the F0 subunit of ATP synthase (Andries 2005). The specificity of the R207910 for mycobacteria could be explained because of the low sequence similarity between the AtpE proteins of mycobacteria and other microorganisms. However since the specificity of other antituberculosis drugs such as INH, ETH, and PZA for mycobacteria, is because these are prodrugs requiring activation by a mycobacterial enzyme, it is possible that R207910 could also be a prodrug, although its chemical structure gives no clues to potential activation sites (Cole 2005).

The compound R207910, now designated as TMC207, is being developed in phase IIa trials for the treatment of active TB. In the established murine model of TB, compound R207910 on its own is as active as the standard regimen (RMP, INH and PZA). Furthermore, when added to RIF, INH, and PZA, R207910 can shorten treatment (Lounis 2006).

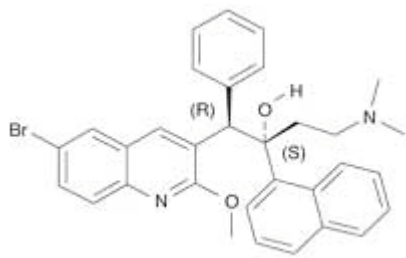


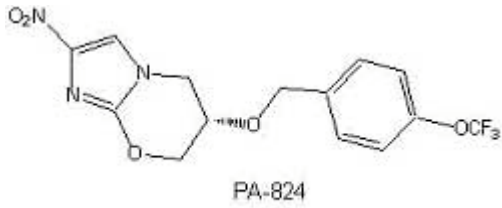
Figure 18-4: Structure of R207910

Nitroimidazoles

A series of bicyclic nitroimidazofurans, originally investigated as radiosensitizers for use in cancer chemotherapy, were found to possess activity against cultures of replicating *M. tuberculosis* and had significant *in vivo* activity in a murine infection model. The lead compound in this series, CGI-17341 was mutagenic, discouraging further investigation of the antibacterial activity of the compound series. These

studies suggested, however, that the bicyclic nitroimidazoles might be potential antituberculosis agents. A series of 328 3-substituted nitroimidazopyrans (NAPs) were synthesized on the basis of the structure of CGI-17341. One NAP compound, PA-824 (Figure 18-5), exhibited a low MIC (0.015 to 0.25 mg/L) against *M. tuberculosis* (Duncan 2003). Multidrug resistant strains exhibited comparable susceptibility to PA-824, indicating that there is no possibly cross-resistance with current antituberculosis drugs. Furthermore, it showed activity against non-replicating *M. tuberculosis* in an anaerobic culture model. In fact, metronidazole, a structurally related antibiotic, used to treat anaerobic infections, possesses activity against static *M. tuberculosis* cells surviving under anaerobic conditions (Stover 2000). In addition, this compound shows no evidence of mutagenicity in a standard battery of genotoxicity studies, no significant cytochrome P-450 interactions, and no significant activity against a broad range of Gram-positive and Gram-negative bacteria (Onyebujoh 2005). Like its progenitors metronidazole and CGI-17341, PA-824 is a prodrug of the nitroimidazole class, requiring bioreductive activation of an aromatic nitro group to exert its antituberculosis effect (Manjunatha 2006). Intriguingly, PA-824 is active under microaerophilic/anaerobic conditions, suggesting that it may have the potential to completely eradicate tissues of *M. tuberculosis.*

Two PA-824 analogues currently under investigation, PA-822 and PA-647, have greater *in vitro* activity than PA-824, but are not as active *in vivo*. Pharmacokinetics may account for the difference between the *in vitro* and *in vivo* activity of the three nitroimidazopyran compounds. Comparative pharmacokinetic studies in rats found that PA-824 had a high degree of tissue penetration and high bioavailability. In contrast, PA-647 and PA-822 have a poor degree of tissue penetration and poor bioavailability. In addition, PA-824 has a longer half-life, and the clearance of PA-822 and PA-647 appears to be faster than that of PA-824. More potent compounds that have better pharmacokinetic parameters hold promise for being more effective *in vivo* than PA-824.



PA-824

Figure 18-5: Structure of PA-824

Recent studies have demonstrated a diversity of sources and strategies for research on new drugs for the treatment of TB. Natural and synthetic sources, through bioassay-guided or screening methods, have been investigated (Ahmad 2006; Ballell 2005; Biava 2006; De Oliveira 2006; Falzari 2005; Hudson 2003; Okunade 2004; Pauli 2005). Besides, strategies such as the identification of new targets using computational software to investigate vital function (Hasan 2006) or the use of genetic tools such as random mutagenesis can help to identify new targets for new anti-TB drugs (Kana 2004).

18.7. Useful links

- Center for Disease Control and Prevention http://www.cdc.gov/nchstp/tb/default.htm
- Global Alliance for Tuberculosis Drug Development http://new.tballiance.org/
- Stop TB Partnership http://www.stoptb.org
- World Health Organization http://www.who.int/topics/tuberculosis/en/

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