Molecular Mechanisms of Resistance to Injectable Agents in Mycobacterium Tuberculosis

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ABSTRACT

Tuberculosis (TB) has remained one of the most difficult infections to treat. Most current TB regimens consist of six to nine months of daily doses of four drugs that are highly toxic to patients. The purpose of these lengthy treatments is to completely eradicate Mycobacterium tuberculosis, notorious for its ability to resist most antibacterial agents, thereby preventing the formation of drug resistant mutants. On the contrary, the prolonged therapies have led to poor patient adherence. This, together with a severe limit of drug choices, has resulted in the emergence of strains that are increasingly resistant to the few available antibiotics. Here we review our current understanding of molecular mechanisms the profound drug resistance of M. tuberculosis. This knowledge is essential for the development of more effective antibiotics that not only are potent against drug resistant M. tuberculosis strains but also help shorten the current treatment courses required for drug susceptible TB.

INTRODUCTION

The emergence of drug-resistant TB in many countries has become a major public health problem and an obstacle to effective tuberculosis control. In 2011, the World Health Organization (WHO) reported nearly 60,000 new cases of multidrug-resistant tuberculosis (MDR-TB) [1] and estimates of the annual global incidence are much higher. The emergence of drug-resistant strains has made the treatment of TB complex, costly, toxic, time-intensive, and less efficacious. Design of a treatment regimen for drug-resistant TB includes the administration of first-line drugs to which the strains remain susceptible together with second-line drugs. These second-line agents are more expensive, more difficult to administer (several require intravenous administration), and are often associated with severe toxicities, including hepatic and renal dysfunction. In comparison to the 6 months required to treat drug-susceptible TB, drug-resistant TB requires a prolonged treatment duration of 18 to 24 months. These logistics constitute considerable hardships for patients as well as for overburdened public health services. Too frequently, premature discontinuation of therapy occurs, leading to treatment failure and the emergence of Mycobacterium tuberculosis strains with additional drug resistance.

Definitions of drug resistant tuberculosis (TB):

MDR-TB is defined as M. tuberculosis with in vitro resistance to two first-line medications: ionized (isonicotinic acid hydrazide, INH) and rifampin (RIF). Extensively drug-resistant TB (XDR-TB) is defined as M. tuberculosis that is resistant to not only INH and RIF, but also to other medication classes that comprise the backbone of drug-resistant TB therapy, namely a quinolone and one of the second-line injectable drugs (kanamycin [KAN], amikacin [AMK], or capreomycin [CAP]) [2]. Totally drug-resistant TB (TDR-TB) refers to strains that are resistant to all available TB drugs, although the number and degree of resistance to each drug.
Mechanisms of drug resistance:

The traditional mechanisms by which bacteria achieve antimicrobial resistance are (i) barrier mechanisms (decreased permeability/efflux), (ii) degrading/inactivating enzymes, (iii) modification of pathways involved in drug activation/metabolism, and (iv) drug target modification or target amplification. As will be discussed below, *M. tuberculosis* uses all of these mechanisms to achieve resistance. For the aim of this article we define drug resistance as genetic changes that alter the phenotypic resistance levels. Antibiotic tolerance (not discussed herein) addresses mechanisms that do not require genetic changes in order to achieve an altered phenotypic resistance level and is usually reversible after removal of the drug or a change in the growth conditions.

Amino glycosides have been used to treat TB since the introduction of the first antituberculous agent, streptomycin (STR). Currently, STR remains a first-line agent, although its use is largely restricted to retreatment cases because like other amino glycosides it must be given parent rally. AMK and KAN are the other major amino glycosides used for TB. These may be given intravenously or intramuscularly and are second-line agents used to treat MDR- or XDR-TB. Amino glycosides are considered bactericidal drugs and achieve rapid bacterial killing during the initiation phase of treatment. They have poor sterilizing activity and must be combined with agents such as rifamycins and pyrazinamide (PZA) in order to achieve durable cure.

Mechanism of action:

Amino glycosides inhibit protein synthesis by binding to the 30S subunit of the mycobacterium ribosome. The majority of mutations that confer amino glycoside resistance lead directly or indirectly to alterations in the amino glycoside binding pockets of the ribosome, which prevent drug binding but preserve ribosome function as occurs with mutations in the rpsL, rrs, and gidB genes. A minor mechanism of amino glycoside resistance is drug modification like that which occurs with the Eis protein, an amino glycoside acetyl transferase.

STR is a streptidine amino glycoside. On binding to the 30S ribosomal subunit, STR inhibits translational initiation and may also cause misreading of mRNA (6). AMK and KAN are deoxystreptamine amino glycosides, which bind to a different locus on the 30S ribosome. Because of these differences in ribosome binding site between STR and AMK/KAN, different drug-conferring mutations are associated with these two drug groups. In contrast, owing to the structural and functional similarity between AMK and KAN, there is extensive cross-resistance between them.

Mechanism of resistance:

Target modification mutations:

**rpsL** gene:

The rpsL gene encodes the 12S protein, which is a structural component of the ribosome. The RpsL protein serves to stabilize the pseudo knot that is formed by the 16S rRNA component of the 30S ribosome. While rpsL is an essential gene, no synonymous mutations are tolerated for ribosomal function but result in reduced amino glycoside binding. Common mutations in amino glycoside resistance that confer resistance to STR are RpsL K43R and K88R [7,8].

**rrs** gene:

The rrs gene encodes the 16S rRNA itself. The 16S rRNA contains key structures such as the 530 stem loop and the 915 turn; both of these rRNA structures form contacts for the binding of amino glycosides. Like rpsL, rrs is an essential gene. No synonymous SNPs in the rrs gene result in reduced amino glycoside binding while allowing for preserved ribosome function. Many bacteria harbor multiple copies of the rrs gene, and consequently single-allele rrs mutations confer low-level amino glycoside resistance. In contrast, *M. tuberculosis* has only one rrs gene, and mutations in the *M. tuberculosis* rrs gene are usually associated with high-level amino glycoside resistance. While more than 20 rrs mutations have been associated with amino glycoside resistance, the common polymorphisms are A1401G, A514C, and C517T. Some rrs mutations also confer resistance to the cyclic peptide antibiotic CAP. The most common mutation for second-line amino glycoside resistance is A1401G, which was found in 78%, 56%, and 76% of AMK, KAN, and CAP resistant strains, respectively, in one large systematic review [9].

**gidB** gene:

The gidB gene encodes a 7- methylguanosine (m7G) methyltransferase that specifically modifies residues on the 16S rRNA (rrs). gidB is a nonessential gene, and loss-of-function mutations in gidB result in failure to methylate G527 within the 530 loop of the 16S rRNA molecule [10]. Reduced ribosomal methylation confers low-level amino glycoside resistance by reducing the affinity of the drugs for the 16S rRNA binding site. Many different gidB mutations including deletions are associated with amino glycoside resistance, suggesting that loss
of function confers resistance. Polymorphisms in gidB have also been identified in drug-susceptible strains, indicating that as for other resistance-conferring loci, the presence of a mutation in a gene is not necessarily indicative of resistance.

**Mechanism of resistance:**

*Inactivating mutations: eis*

The eis gene encodes an aminoglycoside acetyltransferase, which has an affinity for KAN. KAN acetylation inactivates the drug by preventing it from binding to the 30S ribosome. Promoter upregulating mutations in the 5’ untranslated region of eis are associated with clinically relevant M. tuberculosis resistance to KAN. While the Eis protein is capable of acetylating AMK, its affinity for AMK is low, and studies of clinical isolates with eis promoter-up mutations reveal selective KAN resistance with relative preservation of AMK susceptibility [11]. Nevertheless, clinical isolates with eis mutations that are KAN and AMK-resistant have been described [7], so the specificity of eis mutations for KAN resistance remains uncertain.

**Mechanism of resistance:**

*Up regulation of drug in activator and drug efflux: whiB7*

Recently, mutations in the promoter region of the transcriptional activator whiB7 have been identified in clinical isolates. These promoter mutations result in up regulation of the whiB7 regulon, leading to an increase in eis expression and resulting KAN resistance [12]. The whiB7 regulon also includes Rv1258c, which encodes an efflux pump, tap, that is upregulated in tandem with eis, resulting in STR resistance. whiB7 promoter mutations therefore can result in both a target modification and efflux mechanism of resistance, leading to cross-resistance to two amino glycoside drug groups [12].

**STR resistance-conferring mutations:**

*rpsL, rrs, gidB*

High-level STR resistance is achieved predominantly by mutations in rpsL (~50% of STR-resistant strains) and rrs (~15% of STR-resistant strains). Important rrs mutations associated with STR resistance are rrs A514C and A908C. While the rrs A514C mutation is associated with high-level resistance to STR, the most common rrs mutation conferring AMK and KAN resistance (rrs A1401G) does not confer STR cross-resistance. gidB mutations are less frequent among STR-resistant strains (~20% of strains) and are associated with low-level STR resistance [7]. Mechanisms of low-level resistance have not been well characterized but probably involve drug efflux.

**Amikacin and kanamycin resistance conferring mutations: rrs, eis**

The rrs A1401G is the most frequent mutation conferring AMK and KAN resistance, occurring in ~85% of strains that are AMK or KAN resistant. The presence of A1401G appears to be 100% specific for co resistance to AMK and KAN. Of AMK and KAN resistant strains, 10 to 15% harbor eis mutations, indicating that eis is a minor determinant of AMK and KAN resistance [5,7].

**Capreomycin:**

CAP and viomycin (VIO) are cyclic peptide antibiotics with structural similarity. The drugs have uniform cross-resistance in M. tuberculosis and appear to have similar mechanisms of action. While VIO is rarely used due to high toxicity, CAP is an injectable drug commonly used as a second-line agent in the management of MDR and XDR-TB that is resistant to amino glycosides.

Like the structurally unrelated amino glycosides, CAP and VIO are bactericidal drugs that inhibit protein synthesis. VIO has been shown to bind both the 30S and 50S ribosome subunits and to inhibit ribosomal translocation by interference with the peptidyl tRNA acceptor site [13,14]. Due to overlap in the binding region of CAP and the amino glycosides, certain mutations confer cross-resistance to CAP and AMK/KAN. In contrast, cross-resistance between CAP and STR is rare. The major mechanisms of CAP resistance are mutations that result in ribosome modification, particularly rrs and tlyA. Interestingly, tlyA mutations uniquely affect CAP resistance and do not appear to play a role in resistance to aminoglycosides.

**Mechanism of resistance:**

*Target modification mutations*

**Rrs:**

The rrs A1401G mutation is found in ~85% of CAP resistant XDR-TB strains. Other rrs mutations including C1402T and G1484T are also associated with CAP resistance [7].

**tlyA:**

The tlyA gene is a nonessential gene found in many bacteria. Transposon mutants of tlyA as well as spontaneous point mutants display significant CAP and VIO resistance. Biochemical, genetic, and comparative
genomics suggest that the tlyA gene is an rRNA methyltransferase and that loss of methyltransferase activity yields an unmethylated ribosome that is resistant to CAP inhibition [15]. This mechanism is similar to that of the eis gene that confers resistance to KAN. Numerous tlyA mutations have been reported including L180R, S265T, S64W, frame shift at 218L, N236K, and L150P [7,15,16].

Conclusion:

It has only been less than 100 years since antibiotics were first used to treat bacterial infections. This time period is very short considering the pre-antibiotic era dated back thousands of years during which infectious diseases might have acted as selective forces of human evolution. For a long time, humans and bacteria had co-evolved in duels. However, everything has changed since antibiotics were discovered and applied in mass amounts to modern medicine: bacteria now must evolve under the additional selective pressure of these killing molecules. Evidence thus far indicates that pathogenic bacteria such as M. tuberculosis are well able to cope with this pressure and that they have evolved to become progressively resistant to antibiotics. Acquired resistances due to mutations in genes encoding target proteins or genes required for drug activities have allowed rapid evolution of mutants that become newly resistant to the antibiotics used. Accumulation of these resistance mutations has led to the emergence of M. tuberculosis strains that are more and more resistant to the available antibiotics. More alarmingly, recent studies indicate that these drug resistant mutants are able to evolve to regain fitness via compensatory mutations, thus enhancing their transmissibility and/or virulence. In addition to the acquired resistances caused by chromosomal mutations, M. tuberculosis is naturally resistant to most antibiotics. The profound intrinsic drug resistance in M. tuberculosis includes both passive and specialized mechanisms, the latter of which are able to respond to the presence of antibiotics. In fact, structural proteins capable of antibiotic resistance might have existed long before the clinical applications of these molecules. While these “drug resistance” proteins might still play roles in the physiology or metabolism of M. tuberculosis and other bacteria, inductive expression upon antibiotic exposure allows activities of these proteins important for antibiotic resistance.

REFERENCE

