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Protein Synthesis Inhibitors

This lecture discusses a diverse group of antibiotics that are grouped together because they all have a common mechanism of action – they are protein synthesis inhibitors. The drug target of these different classes of drugs often differs (although some overlap), however all affect protein assembly at the ribosomal level. The different classes of antibiotics are the following: macrolides/lincosamides, aminoglycosides (the only bactericidal class in this group), tetracyclines, chloramphenicol, streptogramins and oxazolidinones. These drugs are rarely the drugs of choice for either a particular infection or for a particular bacterial species however they continue to have an important role as antibacterial agents. A general review of protein synthesis (translation) is shown in the first few power point slides.

Macrolides/Lincosamides

Introduction: The macrolides are a family of safe, bacteriostatic drugs that are generally used in the treatment of community acquired infections. Erythromycin is the original agent and continues to be used in different iterations. A second generation of these agents referred to as azalides have an extended spectrum of activity as well as different pharmacokinetics. Lincosamides are limited to clindamycin, a drug primarily utilized for its anaerobic activity. It will not be discussed further.

Chemical Structure: Large 14-16 member lactone ring structure

Mechanism of Action: Binds to the 50S ribosomal subunit reversibly blocking the binding of tRNA to the acceptor site. This blocks translocation of the peptide chain. Because in vitro studies also show macrolides interfering with binding of other protein synthesis inhibitors it is believed that these drugs (*e.g.*, chloramphenicol) have overlapping binding sites

Mechanism of Resistance: Resistance has limited the use of this class of drugs recently. Bacteria may be macrolide resistant due to a permeability barrier (enterobacteriaceae). Active efflux pumps can expel the drug in Gram positive species. A single step mutation in the 50 rRNA gene can confer high level macrolide resistance in some species. Finally modification of the 50S rRNA subunit decreases binding of erythromycin. This can be a plasmid mediated function.

Antibacterial spectrum: Macrolides are active against Gram positive bacteria including streptococci, pneumococci, methicillin sensitive staphylococci. The limited Gram negative spectrum includes bordetella and campylobacter. The macrolides also have activity against mycoplasma, legionella, chlamydia and the treponemes. Azalides have an increased spectrum that includes activity against hemophilus species, *Mycobacterium avium intracellulare* (MAI) and depending on the azalide increased activity against Gram positives (clarithromycin) or Gram negatives (azithromycin)

Pharmacology: Macrolides are generally given orally. Because they are not acid stable they are generally prepared with an acid resistant coating. Intravenous preparations are available for more serious infections. The $t_{1/2}$ is 1.4h and for the azalides this is considerably extended allowing for single or twice daily therapy. This family of agents achieves good distribution and, of note, achieves high intracellular concentrations (especially the azalides).

Toxicity: Toxicity is limited. GI symptoms (due in part to the role of macrolides as a motilin receptor agonist) are more common with macrolides than azalides.

Indications for use: Community-acquired pneumonia – mycoplasma, legionella, chlamydia infections; pertussis, campylobacter gastroenteritis and MAI. There is an investigational role in the use of these drugs as anti-inflammatory agents in cystic fibrosis and in the prevention of atherosclerotic cardiovascular disease.

Aminoglycosides

Introduction: The aminoglycosides are bactericidal agents that are used for the treatment of serious infections. They are not used as single agents because of concerns about the emergence of resistance. In addition they have a very narrow therapeutic to toxic ratio (*i.e.* the serum level needed to be effective as a therapeutic agent is close to the level where drug toxicity is encountered).

Chemical Structure: Consist of at least two amino sugars linked by glycosidic bonds to an aminocyclitol ring

Mechanism of Action: A rapidly bactericidal agent with multiple mechanisms of action. The primary target is the 30S ribosome causing premature chain termination and RNA codon misreading. In addition causes leakage of the outer membrane of Gram negatives. The basis for the bactericidal activity is not completely understood.

Mechanism of Resistance: The principal means of resistance is aminoglycoside modifying enzymes. These enzymes such as the adenyltransferases or phosphotransferases modify the aminoglycoside rendering them ineffective. The genes for these enzymes are often plasmid borne and as a result are readily transferred among different bacterial species. An additional mechanism of resistance is diminished uptake of the aminoglycoside via mutations in the electrochemical gradient.

Antibacterial spectrum: Aminoglycosides have a somewhat limited spectrum of activity. They are active against Enterobacteriaceae, pseudomonas species, and Gram positive bacteria including staphylococci and streptococci. Some of the aminoglycosides are active against mycobacteria. They have no activity against anaerobes.

Pharmacology: Aminoglycosides are primarily used for parenteral therapy. There is reasonable distribution into tissues because of its low protein binding and water solubility. Penetration across the blood brain barrier is poor as is penetration into bronchial secretions. Aminoglycosides are excreted virtually unchanged in the urine.

Toxicity: These drugs have significant toxicity. This includes nephrotoxicity in 5-25% of patients, ototoxicity including both cochlear and vestibular damage, and, infrequently, neuromuscular blockade. To reduce the risks of these complications drug levels are generally monitored in subjects with renal disease.

Indications for use: Empiric combination therapy for life-threatening infections. Used as combination therapy for resistant bacterial infections, and combination (synergy) therapy for enterococcal infections that require bactericidal activity.

Tetracyclines

Introduction: The tetracycline class of antibiotics is a large family of broad spectrum, bacteriostatic agents that have a limited but important role in the armamentarium of antibacterial agents. The first tetracycline was the product of a soil-screening program to detect antimicrobials in the 1950's.

Chemical Structure: The basic structure consists of four fused 6-carbon rings. Activity of different members of the class created by modification at selected carbon sites.

Mechanism of Action: The primary target is the 30S ribosome, preventing binding of aminoacyl tRNA to the acceptor (A) site on mRNA.

Mechanism of Resistance: The primary mechanism of resistance to tetracyclines is decreasing penetration or increasing export of the drug via an efflux pump. This mechanism may be plasmid mediated and also confers resistance to all members of the class.

Antibacterial spectrum: Although these drugs have Gram positive activity, including pneumococcus, streptococci and enterococci, they are rarely used because of resistance. They have activity against Gram negatives – *E. coli*, but not other Enterobacteriaceae, neisseria, hemophilus and some shigella. They are also active against mycoplasma, rickettsia, legionella, spirochetes and chlamydia.

Pharmacology: Most tetracyclines are administered orally. There are both short and long acting agents. Most are lipophilic and have excellent tissue distribution. The most commonly used tetracyclines, minocycline (metabolized in the liver) and doxycycline (inactivated in the intestine), are cleared by nonrenal routes.

Toxicity: Although generally well tolerated, there is a long list of potential side effects. These include photosensitivity, discoloration of children's teeth, hepatotoxicity and hypersensitivity reactions.

Indications for use: Their principal role is for treatment of lyme disease, community- acquired pneumonia and acne.

Chloramphenicol

This drug is primarily of historical interest, however because it continues to be used with some frequency in the developing world it should be mentioned. It is a broad spectrum agent that is a single member of its class. It achieves high concentrations following oral administration including in the CSF. It has activity against agents that cause meningitis including pneumococcus, hemophilus and neisseria. It binds to the 50S ribosome preventing attachment to the acceptor (A) site on mRNA. Chloramphenicol causes a dose related marrow depression and rarely aplastic anemia that is idiosyncratic. In neonates the Gray baby syndrome was a concern.

Streptogramins

This is a combination of two drugs derived from pristinamycin. The first is quinupristin and the second is dalfopristin. Both are protein synthesis inhibitors working at the 50S ribosomal subunit. This combination is used exclusively to treat resistant Gram positive infections including vancomycin resistant enterococci (only *E. faecium*) and complicated methicillin resistant *S. aureus* (MRSA) infections where vancomycin cannot be used. Resistance has occurred primarily by ribosomal modification (methylation). This can be plasmid mediated. The drug is bactericidal

if the isolate is macrolide susceptible. It is only available parenterally. Toxicity includes phlebitis at the infusion site and myalgias which can be quite bothersome.

Oxazolidinones

Linezolid is the sole member of this new class of bacteriostatic agents. This family of drugs is active at the 50S ribosomal subunit. It inhibits formation of the initiation complex. It is active against Gram positive bacteria including MRSA and all vancomycin resistant enterococci. It has the pharmacological advantage of being available both orally (with excellent absorption) and parenterally. Resistance has been described occurring by mutation of the ribosomal binding site. The drug is well distributed and well tolerated. Thrombocytopenia and myelosuppression have occurred with prolonged administration (> two weeks therapy).

Protein Synthesis Inhibitors

Drug	Mechanism of Action	Mechanism of Resistance	Spectrum of Activity	Pharmacology	Indications for Use	Toxicity
Macrolides • erythromycin **Azalides** • azithromycin • clarithromycin	Inhibits formation of 50S ribosome blocking transpeptidation or translocation.	• decreased permeability of bacterial envelope to macrolide • chromosomal mutation of 50S ribosomal protein • active efflux of antibiotic - plasmid-mediated • enzymatic inactivation by esterases or phosphotransferases - plasmid mediated	broad-spectrum bacteriostatic **Erythromycin** • Gram-positive: pneumococci, viridians, streptococci, Group A streptococci, methicillin-sensitive staphylococci • gram-negative: bordetella and campylobacter infections; rarely used for neisseria infections • mycoplasma, legionella, chlamydia, treponemes **Azalides** • spectrum similar to erythromycin • increased activity against hemophilus species • increased activity against *Mycobacterium avium intracellulare* (MAI), toxoplasma • azithromycin - enhanced gram-negative activity • clarithromycin - enhanced gram-positive activity	• primarily oral, limited parenteral use • well-absorbed, esp. azalides • erythromycin $t_{1/2}$ - 1.4h • azalides have longer $t_{1/2;}$ can be taken 1-2x a day • well-distributed; good levels in CNS w/ inflammation • **achieves high conc. in alveolar cells & PMN leukocytes, esp. the azalides** • most drug metabolized by glucuronidation in liver; excreted in inactivated form	• treat community acquired pneumonia b/c activity against mycoplasma, legionella, chlamydia • treat pertussis, bordetella infections • treat *Campylobacter jejuni* gastroenteritis • treat MAI (azalides) • treat Bacillary angiomatosis • alternative agents for: group A, C, G, streptococcal infections, rheumatic fever prophylaxis when penicillin is contra-indicated, C. *trachomatis* urethritis (azalides only req. single pill) • investigational uses of macrolides: prevent atherosclerotic cardio-vascular disease, potential anti-inflammatory in Rx of *P. aeruginosa* infections in cystic fibrosis	• well-tolerated • GI symptoms – cramps, diarrhea secondary to motility stimulating effects of antibiotic. • cholestatic hepatitis (rare) • drug-drug interactions – erythromycin > clarithromycin o may interfere with cytochrome P450 enzymes leading to ↑ levels of other drugs, e.g. dilantin, warfarin, cyclosporine
Lincosamide • clindamycin	mechanism of action similar to macrolides		bacteriostatic • activity against anaerobic bacteria, toxoplasma • treat osteomyelitis – achieve high bone conc. • active against staphylococci		• primarily to treat anaerobic infections	• frequent diarrhea • allergy

Drug	Mechanism of Action	Mechanism of Resistance	Spectrum of Activity	Pharmacology	Indications for Use	Toxicity
Aminoglycoside	primary site of action – interferes with mRNA translational accuracy at 30S ribosome → misreading and premature chain termination binds to and alters bacterial cell membrane cause leakage and disruption of cell wall	• quite common with aminoglycosides • primary mechanism - enzymatic modification of the aminoglycoside by adenylation, phosphorylation or acetylation (enzymes on plasmids or transposons, often in concert with Gram-positive beta-lactamase) • anaerobes are completely resistant b/c lack an oxygen-dependent transport system • chromosomal mutations	narrow-spectrum <u>bactericidal</u> • active against aerobic gram-negative bacilli, including *P. aeruginosa* • primarily used for gram-positive synergy • selected aminoglycosides have activity against mycobacterial species, *Yersinia pestis* • no activity against: hemophilus, anaerobes, pneumococcus, neisseria	• minimal absorption after oral admin • limited tissue distribution due to polarity • not metabolized, excreted almost entirely by the kidney • rapid absorption after IM administration; cruel but sometimes necessary • aminoglycosides cause concentration-dependent as opposed to time-dependent killing (e.g. beta-lactams)	• empirical therapy: life-threatening infections that require broad spectrum coverage • specific therapy: synergistic antimicrobial activity for enterococcal endocarditis and pseudomonas infections • rarely have monotherapy with aminoglycoside except as inhalation therapy for cystic fibrosis patients with pseudomonal pneumonia because delivers higher conc. of drug	• significant nephrotoxicity: incidence 5-25% - damages proximal tubular cells. Need to carefully monitor use o changed admin: small dose, 3x per day → one large dose, 1x/day • ototoxicity o monitor levels for vestibular toxicity; may cause irreversible damage • neuromuscular blockade: rare
Tetracyclines	binds to 30S portion of ribosome and prevents access of aminoacyl tRNA molecules to the mRNA ribosome peptide complex	• common in both Gram-positive and Gram-negative bacteria • generally, but not exclusively, plasmid-mediated • decreased uptake and increased excretion of the drug (efflux pump); resistance is conferred to all tetracyclines • associated with extensive use in animal food	broad spectrum <u>bacteriostatic</u> • very broad spectrum but wide spread resistance o Gram-positives: *S.pneumoniae, S.pyogenes, S.agalactiae*, enterococci o Gram-negatives: *E .coli, Neisseria spp., Hemophilus spp., Shigella spp.* • miscellaneous pathogens - Spirochetes – Borrelia, Rickettsiae, Chlamydia, Mycoplasma	• primarily oral agents • cations Ca^{2+} and Mg^{2+} (often found in dairy products) decrease absorption and plasma levels by chelating tetracyclines • half-life varies with agent as does extent of excretion by the kidney • excellent tissue distribution • concentrated in bile; levels of 10-26% of serum in CSF, esp. w/ inflammation	• treatment of chlamydia, mycoplasma, brucella, vibrio, helicobacter, rickettsia, borrelia, ehrlichia infections • *Mycobacterium marinum* infections • acne – tetracycline in low doses • rarely the first drug of choice except in borrelia and ehrlichia infections	• generally well-tolerated • GI symptoms are common • **photosensitivity** major concern • hypersensitivity reactions: primarily rash; urticaria, anaphylaxis (rare) • hepatotoxicity – seen especially during pregnancy so should avoid

Drug	Mechanism of Action	Mechanism of Resistance	Spectrum of Activity	Pharmacology	Indications for Use	Toxicity
Streptogramins combination drug that derived from pristinamycin: 30% quinupristin 70% dalfopristin	both interfere with 50S ribosomal subunit, different target site quinupristin inhibits peptide chain elongation dalfopristin interferes with peptidyl transferase	• primarily occurs by methylation of MLS binding site; plasmid-mediated; cross-resistance occurs in that methylation of MLS site will cause resistance to all three families of protein synthesis inhibitors • also have drug modification or efflux pump (less common)	limited spectrum <u>bactericidal</u> • Gram-positive cocci – staphylococci, streptococci, and enterococci (only *E. faecium* not *E. faecalis*)	• Only parenteral • well-distributed • metabolized by liver to inactive metabolites	• primarily used when have vancomycin-resistant enterococcus – 3rd line drug of choice for enterococcus after ampicillin and vancomycin • similarly used for methicillin-resistant staphylococci – 3rd line drug of choice	• myalgias • arthralgia • phlebitis at site of administration
Oxazolidinone • linezolid	binds at 50S ribosomal subunit and inhibits formation of initiation complex	• resistance by mutation of 23S RNA (50S subunit) • resistance described in enterococci and staphylococci • does not, at present, exhibit cross-resistance with other protein synthesis inhibitors	• <u>bacteriostatic</u> synthetic antibiotic • activity against streptococci, pneumococci, enterococci (active against both types of enterococci species), and staphylococci	• well-tolerated • advantage over streptogramins - can be administered orally or parenterally • excellent distribution, metabolized by liver, excrete by urine	• used in setting where have methicillin, penicillin, and vancomycin-resistance • treatment of drug-resistant enterococcal or staphylococcal infections	• myelosuppression - thrombocytopenia most common develops with prolonged administration of drug. Need to monitor blood count