Georg Domagk and colleagues at Bayer spent 5 years screening dye compounds for antibacterial activity.

Thought Prontosil was the active drug, in fact it is metabolized to sulfanilamide, which is active, and had been discovered years before but not appreciated as an antibacterial.

Sulfanilamide is simpler and cheaper to synthesize, and was modified to make subsequent sulfa drugs such as sulfmethoxazole.

Sulfonamides are competitive inhibitor for para-aminobenzoic acid (PABA) in the biosynthesis of folic acid.

Depleting folic acid hinders the eventual production of DNA so bacteria are unable to reproduce.

Often given with trimethoprim (e.g., SMX/TMP), synergistic.

  - Alone, each is bacteriostatic, together bactericidal.
Sulfonamide antimicrobials

- Mammalian cells do not synthesize folate, they actively import dietary folate
- Bacteria do not take up folate, they make it

\[ \text{Sulfonamide} \rightarrow \text{PABA} \rightarrow \text{Dihydrofolate} \]

- SMX is a PABA analog that binds to dihydropteroate synthase (synthetase) and prevents it from using PABA
- TMP binds to and inhibits dihydrofolate reductase
- Both those enzymes are needed by most bacteria to synthesize tetrahydrofolate (THF)
- Inhibition of THF synthesis, prevents synthesis of thymidine (i.e. the nucleoside “T”), which is required for DNA synthesis
- Inhibition by TMP/SMX (1:5 ratio) is synergistic
  - Alone, each drug is bacteriostatic, together bactericidal
  - If a bacteria is resistant to one of the two, lose synergy

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image from wikimedia commons
Spectrum of activity

- Not very effective against anaerobes, some oral anaerobes

- Active against many Gm+ and Gm- aerobes, for example:
  - *S. aureus* including some use against CA-MRSA
  - *H. influenzae*
  - Salmonella
  - *E. coli*: UTI
  - *Proteus mirabilis*: UTI

- Nosocomial pathogens:
  - *Burkholderia cepacia*: e.g. pneumonia in immunocompromised patients, CF
  - *Stenotrophomonas maltophilia*: respiratory tract infections, UTI, from device insertions (tubes, cath)
  - *Serratia marcescens*: UTI, bacteremia from catheter insertions

- Protozoal and fungal parasites:
  - *Toxoplasma gondii*: toxoplasmosis
  - *Pneumocystis jiroveci*: pneumonia, esp in HIV patients

TMP-SMX in the body

- 85% bioavailable, not affected by food

- Broad distribution in tissues
  - TMP is more lipophilic, so it gets concentrated to higher levels in tissue than SMX, so 1:5 (SMX:TMP) ends up ~1:20, which is optimal for synergy

- Both penetrate to CSF

- SMX gets acetylated in the liver, TMP is excreted in urine unchanged
TMP-SMX resistance

- Fairly wide-spread resistance due to frequent use of the treatment
- Target enzyme mutations to reduce drug binding
  - Dihydropteroate synthase
  - Dihydrofolate reductase (e.g. in E. faecalis)
- Active efflux
  - *Pseudomonas aeruginosa*
- Some have natural level of resistance because they do not synthesize their own folic acid
  - *E. faecalis*

Metabolism and toxicity

- “Slow acetylators” have a higher risk of developing toxicity (pathway on the right), lower clearance
- Patients deficient in enzyme G6PD higher risk of developing hemolytic anemia due to reduced ability to regenerate glutathione (GSH)
TMP-SMX adverse effects

- **Crystallurea**
  - Metabolized sulfonamides are insoluble and form crystals in urea
  - Maintain hydration

- **Kernicterus**
  - Brain damage due to jaundice (accumulation of bilirubin)
  - Sulfonamides displace bilirubin from binding to albumin
  - Not to be used in patients < 2 months old

- **Should not be given to patients who are folate deficient or pregnant:** TMP may interfere with folate metabolism (DHFR enzyme)

- **Rash, nausea, vomiting**

- **Steven-Johnson Syndrome**
  - A severe skin reaction, layers of skin separate

- **Many of the adverse reactions to TMP-SMX are much higher in frequency in HIV-infected individuals (25-50%) vs (6-8%)**

TMP-SMX adverse effects

- **Drug interactions, for example:**
  - warfarin
  - cyclosporin
  - rifampin
  - dapsone
  - phenytoin
  - etc.
Products

- **Trimethoprim/Sulfamethoxazole** (1:5 ratio TMP:SMX, “trim-sulfa”, co-trimoxazole)

- Other examples of sulfonamides for protozoal infections:
  - Sulfadiazine or Sulfadoxine-pyrimethamine (pyrimethamine is another drug used to treat protozoal infections)
    - *Plasmodium falciparum* (malaria) if chloroquine resistant
  - Sulfadiazine-pyrimethamine
    - *Toxoplasma gondii*
  - Dapsone: *only in M. lepra* high affinity for PABA binding site
    - *Mycobacterium leprae* (leprosy)

**TMP-SMX summary**

- Combination of two synergistic agents that inhibit bacterial folic acid synthesis
- Fairly broad spectrum Gm+, Gm-, protozoa, pneumocystis
- Good oral bio-availability
- A number of adverse effects to be aware of:
  - Significantly higher rates of adverse effects in HIV+
  - A number of drug interactions, may require monitoring
Macrolides

- All “macrolides” are based on a large, macrocyclic ring +2 sugars (except telithromycin)

- Frequently used for community-acquired respiratory infections; also for skin, otitis media (ear)

- Alternative drug for *Strep.*, *Staph.*, *H. flu* infections for pen-allergic

- Erythromycin isolated 1952 from a soil microbe *Streptomyces erythreus*

- Later drugs (azithro-, clarithro-, telithro-) have broader spectra, especially Gm-, better acid stability and oral bioavailability:

- Excellent tissue penetration, especially azithromycin and clarithromycin, but not to CNS

- Target the 50s large ribosomal subunit in bacteria, inhibit protein synthesis
  - Similar MOA to clindamycin and chloramphenical (shared binding site)
  - Bacteriostatic, except at very high concentration can be bactericidal
**Macrolides MOA**

**30s**

- spectinomycin
- tetracycline 1
- pactamycin
- hygromycin B
- streptomycin
- paromomycin
- genetricin
- tetracycline 2

**50s**

- thioestrepton
- avilamycin
- streptogramin A
- chloramphenicol
- puromycin
- pleuromutilins
- streptogramin B
- lincosamides
- macrolides

**Aminoglycosides**

- tetracyclines

**Induction**

- **MACROLIDES**: Bind to 50s subunit:
  - Induce premature dissociation of peptidyl-tRNA from ribosome, hence premature termination
  - Prevent addition of residues onto nascent polypeptide by blocking A to P translocation

**Inhibition of bacterial protein synthesis**

- **A**: aminoacyl-tRNA
- **P**: peptidyl-tRNA
- **E**: free, exiting tRNA

**Large subunit (50s):**

- Peptide chain synthesis

**Small subunit (30s):**

- mRNA codon, anticodon pairing

- Ribosome: a protein/RNA ribozyme, functions like a machine with moving parts and substrates including mRNA, tRNA, amino acids, cofactors, the nascent polypeptide

[Figure by Stephen Douthwaite, University of Southern Denmark]

[2wdk+2wdl PDB coordinate-based illustrations by David Goodsell]
Resistance

• Resistance is on the rise
  • E.g. between 1995 and 1999, *S. pneumo* resistance to macrolides rose from 10.6 to 20.4%

• Methylation of ribosome binding site via *erm* (erythromycin ribosomal methylation) enzymes
  • Affects erythro-, clarithro-, azithro-, but NOT telithromycin
  • *Erm* expression is inducible

• Esterases cleave the lactone ring

• Active macrolide efflux pumps

• Intrinsic *Enterobacteriacea* resistance due to outer membrane permeability

General spectrum of activity

• Gram+
  • *S. pneumoniae*, *S. pyogenes*
  • *S. aureus*: MSSA but not MRSA

• Gram-
  • *H. influenzae* (not erythromycin), *M. cattarhalis*
  • Clarithromycin and azithromycin more potent than erythromycin

• “Others”
  • *Mycoplasma pneumoniae* (walking pneumonia; a very persistent cough)
  • *Legionella pneumophila* (Legionare’s disease)
  • *Chlamydia trachomatis* (STD) and *Chlamydia* (aka *Chlamydophilia*) pneumoniae
  • *Mycobacterium avian complex* (MAC): Clarithro- and azithro-
Distribution in the body

• Excellent tissue penetration
  - Tissue: blood ratio 10-100:1 so the drug goes to tissue and persists there T1/2~2-4 days
  - Penetration into host cells too, so good for intracellular parasites such as Chlamydia

• Oral bioavailability
  - Erythromycin not acid stable (~25% orally bioavailable); empty stomach
  - Clarithromycin and azithromycin more stable (~50% orally bioavailable); w/ food OK
  - Telithromycin more stable (~57% orally bioavailable); w/ food OK

Erythromycin

• Not acid stable, poor oral bioavailability ~25%

• Good Gm+ and reasonable Gm- activity
  - Staph. aureus, but not MRSA
  - S. pyogenes
  - M. catarrhalis
  - H. influenzae
  - N. menengitidis
  - Mycoplasma
  - Chlamydia
  - Not Enterobacteriaceae, cannot penetrate outer cell membrane
  - Not Pseudomonas

• Uses:
  - URI, LRI: mild to moderate
  - Skin: mild to moderate due to S. pyogenes
  - Diphtheria
  - Amebiasis
  - N. gonorrhea: for pen-allergic
  - Leigonare’s disease
**Erythromycin**

- GI discomfort, diarrhea (13-32%)
  - Induces peristalsis
- Possible risk of QT elongation, arrhythmias
- Drug interactions:
  - P-glycoprotein inhibitor: interacts with digoxin
  - CPY3A4 inhibitor: interacts with carbamazepine, cyclosporin
  - CYP1A2 inhibitor: interacts with theophyline and caffeine
- PO, IV, topical, opthalmic

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**Clarithromycin**

- Acid stable; oral bioavailability ~50% (metallic taste); PO only
- 2-4x more potent than erythromycin
- Broader spectrum of coverage compared to eryth.
- Macrolide of choice for treatment of:
  - *Mycobacterium avium complex* (MAC); common opportunist in AIDS
  - *H. pylori*
- Uses:
  - Phyaryngitis/tonsilitis
  - Acute maxillary sinusitis
  - Bronchitis
  - CAP (community-acquired pneumo)
  - Skin
  - Otitis media
- Precautions:
  - *Pregnancy category C* (risk cannot be ruled out), safety in children < 6 mo. not established. Teratogenic effects observed in animal models.
Azithromycin (Zithromax®)

- Acid stable; oral bioavailability ~50%
- More potent than erythromycin
- Better Gm- coverage than clarithromycin, but less Gm+ than clarithromycin
- Alternative to clarithromycin for MAC
- Macrolide of choice for:
  - *Chlamydia trachomatis*: 1gm single dose
  - *Chlamydia pneuomo., M. cat, H. flu*
  - *N. gonorrhea* (if patient is beta-lactam allergic)
  - *Legionella*

Azithromycin (Zithromax®)

- Does not appear to affect liver function or other drug metabolism
- Precautions:
  - Do not take with antacids, impairs absorption
  - Rare hepatotoxicity
  - Pregnancy category B

- Uses: even for some tougher infections
  - Chronic obstructive pulmonary disease (COPD)
  - CAP
  - Bronchitis
  - STD involving *Chlamydia*
  - *Skin*
  - *Otitis media*
  - *MAC*
Telithromycin (Ketek®)

- Binds more tightly to bacterial ribosome, and in more than one site
- Bugs that are erythromycin resistant (and clarith-, azith- too) may be sensitive to telithromycin
- Does not induce expression of \( \text{erm} \) (erythromycin ribosome methylase) that methylate parts of ribosomal RNA to reduce binding of other macrolides
- Similar spectrum to azithromycin, also covers PRSP, but not MRSA
- Potentially fatal liver toxicity found after it was on the market
- Aggravates myesthenia gravis: autoimmune disorder in which antibodies block nerve/muscle signaling. Lead to a FDA black box warning
- Pregnancy category C

Telithromycin (Ketek®)

- Inhibits liver enzymes: drug-interactions prevalent
- Blurred vision
- Prolonged QT interval: can produce ventricular arrhythmias; avoid in patients with congenital elongated QT, on going arrhythmic conditions, and those taking antiarrhythmic agents

- Acid stable, oral bioavailability \(~57\%\); PO only
- Highest tissue penetration; even higher than azithromycin and clarithromycin
  - E.g. 200-400x above serum concentrations in some lung cells and macrophages
  - Drug stays above MIC for most respiratory pathogens
- Due to potentially severe adverse effects, telithromycin not to be used for mild cases or to treat bronchitis, sinusitis
Macrolide summary

- Erythro-, Clarithro-, Azithro- commonly used for community-acquired respiratory infections
- Shut down protein synthesis by binding to bacterial 50s ribosomal subunit
- Newer macrolides (Az, Cl, Tel) are more acid stable and have a broader spectrum of coverage and greater potency than erythromycin.
- Variety of drug-interactions for Er, Cl, Tel but NOT Az
- Resistance due to change of target binding site (methylation of RNA) and efflux pumps in some bacteria
- Telithromycin to be used with caution, numerous adverse effects