Erythromycin

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Learning Objectives

● Antibiotic Resistance
● Polyketides as drug candidates
Introduction to Erythromycin

- Broad-spectrum (Penicillin)
- Macrolide Class
- Erythromycin A/B/C/D
- Erythromycin ethylsuccinate (EES)

- 1949 Streptomyces erythreus in Iloilo
- 1952 Ilosone Launched
- 1953 Patent Granted
Introduction to Erythromycin

- Lung infections
- Skin infections
- STDs
- Eye infections, ophthalmia neonatorum
- Improved Gastric Emptying*

Effective against:
- Staphylococcus aureus
- Streptococcus pneumoniae
- Streptococcus pyogenes
- Legionella pneumophila
- Neisseria gonorrhoeae
- Mycoplasma and Ureaplasma

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Inhibitory concentration (mcg./ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrococcus pyogenes var. aureus</td>
<td>0.8 (ag)</td>
</tr>
<tr>
<td>Micrococcus pyogenes var. aureus (Penicillin-resistant)</td>
<td>0.4 (ag)</td>
</tr>
<tr>
<td>Micrococcus pyogenes var. aureus (streptomycin-resistant)</td>
<td>0.8 (ag)</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>0.31 (bd)</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>0.2 (ag)</td>
</tr>
<tr>
<td>Vibrio cholera</td>
<td>0.63 (bd)</td>
</tr>
<tr>
<td>Mycobacterium sp. (607)</td>
<td>6.2 (ag)</td>
</tr>
<tr>
<td>Mycobacterium phlei</td>
<td>0.8 (ag)</td>
</tr>
<tr>
<td>Hemophilus pertussis</td>
<td>1.6 (ag)</td>
</tr>
<tr>
<td>Mycobacterium avium</td>
<td>6.2 (ag)</td>
</tr>
<tr>
<td>Mycobacterium smegmatis</td>
<td>6.2 (ag)</td>
</tr>
<tr>
<td>Brucella melitensis</td>
<td>1.56 (bd)</td>
</tr>
<tr>
<td>Brucella suis</td>
<td>1.56 (bd)</td>
</tr>
<tr>
<td>Neisseria intracellular</td>
<td>5.0 (bd)</td>
</tr>
<tr>
<td>Diplococcus pneumoniae (sulfa-fast strain)</td>
<td>0.02 (bd)</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>0.02 (bd)</td>
</tr>
<tr>
<td>Hemophilus influenzae</td>
<td>1.25 (bd)</td>
</tr>
<tr>
<td>Streptococcus viridans</td>
<td>0.16 (bd)</td>
</tr>
<tr>
<td>Corynebacterium diphteriaiae</td>
<td>0.02 (bd)</td>
</tr>
<tr>
<td>Vibrio comma</td>
<td>6.25 (bd)</td>
</tr>
</tbody>
</table>
Introduction to Erythromycin

- Available in the U.S. by Rx only
- Forms
  - Enteric-coated tablets
  - Slow-release capsules
  - Ophthalmic solutions
  - Oral solutions
  - Dermatological solutions
  - Injectable solutions

- Names
  - Robimycin
  - E-Mycin
  - E.E.S.
  - Erymax
  - Erythrocut
  - Erythroped
  - Ilosone
  - Pediamycin
  - Zineryt
  - Abboticin
  - Erycin
  - Stiemycine
  - Acnasol
  - Tiloryth
Antibiotic Resistance

- Microorganism subpopulations become capable of surviving exposure to antibiotic agent
- Major 21st century public health concern
- Overuse of Abx in humans and animals
- Multi-Drug Resistance (MDR)
- Methicillin-resistant Staphylococcus aureus (MRSA)
  - Britain - 1961
  - 4% of fatal sepsis - 1991
  - 37% of fatal sepsis - 1999
  - Now 50% of all Staph. infections in U.S. are resistant to penicillin, methicillin, tetracycline, and erythromycin
Antibiotic Resistance

- **Selective Pressure:** Survivors make fully resistant colonies
- **Mechanism of introduction**
  - Point Mutations (rare)
  - Horizontal Gene Transfer
    - Transduction
    - Transfection
    - Conjugation
- **Mechanism of action**
  - Drug inactivation
  - Alteration of target site
  - Alteration of metabolic pathways
  - Reducing drug accumulation
Antibiotic Resistance

- Macrolide resistance
  - Primarily post-transcriptional methylation of 23S bacterial ribosomal RNA
  - Target site alteration, confers resistance to macrolides, lincosamides, and streptogramins (MLS-resistance).
  - Secondarily:
    - Production of drug inactivating esterases/kinases
    - Production of ATP-dependant efflux proteins
Antibiotic Resistance

Antimicrobial Susceptibility Testing (AST)
○ Grade strain/antibiotic relationship as “Susceptible”, “Intermediate”, or “Resistant”
○ Dilution Technique
  ■ Minimum inhibitory concentration (MIC)
  ■ Predictory of efficacy for variable dose compartments
  ■ MIC for Susceptible S. aureus w/ Erythromycin: 0.12-0.5mg/mL
○ Diffusion Technique
  ■ Paper disc on bacterial lawn
  ■ Zone diameter for Susceptible S. aureus w/ Erythromycin: 22-30mm
Erythromycin Chemistry

- Member of the polyketide class of natural products
  - 20% of top selling drugs
- First and only synthesis reported in 1981 by Nobel laureate Robert B. Woodward at Harvard University
- Produced in large yields by the bacteria Saccharopolyspora erythraea
Biosynthesis of Erythromycin

- Polyketides are synthesized by polyketide synthases (PKSs)
  - Large multi-domain enzymes
  - Separated into functional units called modules
  - Each module adds to growing chain

- Two major steps:
  1) Synthesis of 6-dEB by DEBS
  2) Post-PKS transformation of 6-dEB into Erythromycin

6-Deoxyerythronolide B (6-dEB)
Precursors: Propionyl-CoA (Starter unit) + 6 (2S)-Methylmalonyl-CoA (Extender unit)

DEBS 1 - DEBS 2 - DEBS 3

Loading molecule - Module 1 - Module 2 - Module 3 - Module 4 - Module 5 - Module 6 - End

Aglycone assembly:

6-Deoxyerythronolide B

TE-catalysed cyclization
A New Approach to Drug Development

- Genetic engineering of PKSs can be used to create vast libraries of new compounds
  - e.g. add or remove enzymes on various modules
- This method has been used to synthesize hundreds of new compounds, some of which have promise as drug candidates
a Natural biosynthetic route

Loading module → Module 1 → Module 2 → AVES 1

AT → ACP → KS → AT → ACP → DH → KR → PKS and post-PKS

Avermectins X–Y:
CH═CH or CH₂−CHOH

b Engineered biosynthetic route

Loading module → Hybrid AVES 1 → Hybrid module 2

AT → ACP → KS → AT → ACP → KE → DH → ER → KR → PKS and post-PKS

Ivermectins (and avermectins)

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Nature Reviews | Microbiology
Erythromycin Binding Site

- Binds to 23S rRNA
  - Part of 50S subunit on bacterial ribosome
- Hydrogen bonding interactions between nitrogenous bases and desosamine side chain on C5
- Lactone ring fits into hydrophobic pocket
Mechanism of Action - Inhibition of Protein Synthesis

- Binds inside nascent peptide exit tunnel of bacterial ribosome near 10 Å-wide constriction
- Peptide can grow ~ 6-8 residues long
  - Elongation ceases once molecular roadblock is reached
- Cessation of peptide synthesis results in disassociation of tRNA from ribosome
Pharmacokinetics of Erythromycin
Pharmacokinetics of Erythromycin

Absorption: Common forms of Erythromycin may be administered orally in pill form or I.V.

Enteric-coated base tablet to deal with stomach acidity.

Absorption occurs mainly in the duodenum although a small portion may be absorbed in the stomach.
Pharmacokinetics of Erythromycin

Distribution: Erythromycin is 90% bound to human serum proteins. 99% for propionyl erythromycin. However, not a serious factor in limiting erythromycin tissue levels.

Significant quantities found in tissues of muscle, colon, prostate, salivary, gallbladder, testicles, cerebrospinal fluids, milk, and middle ear exudates.
Pharmacokinetics of Erythromycin

Distribution: Table on right shows serum and ultra-filtrate concentrations of 500 mg oral tablets of erythromycin and propionyl erythromycin.

Serum protein binding: cited for its role in limiting microbiological activity.

Only rate limiting if protein binding irreversible or rate of release ex. slow.
Pharmacokinetics of Erythromycin

Distribution: The table on the right shows erythromycin concentrations in various tissues after single dose of drug administration.

Suggests that propionyl ester doesn’t diffuse as readily.

Repeated dose administration reduces this effect.
Pharmacokinetics of Erythromycin

Metabolism: Occurs in the liver by the hepatic enzyme CYP3A4.

Serum half-life is approximately 1.6 hours in normal subjects.
Pharmacokinetics of Erythromycin

Excretion: Extensively metabolised, therefore only about 5% of the active form is present in urine.

Also excreted in the form of bile.
Pharmacokinetics of erythromycin after the administration of intravenous and various oral dosage forms to dogs

Published 9 May 2008
Mean and Standard Deviation Erythromycin serum concentration-time profile
Pharmacokinetic parameters calculated for each route and formulation summarized

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>L.v. administration (10 mg/kg) (mean ± SD)</th>
<th>Oral estolate tablets (25 mg/kg) (mean ± SD)</th>
<th>Oral ethylsuccinate suspension (20 mg/kg) (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>6.64 ± 1.38</td>
<td>0.30 ± 0.17</td>
<td>0.17 ± 0.09</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>–</td>
<td>1.75 ± 0.76*</td>
<td>0.69 ± 0.30*</td>
</tr>
<tr>
<td>$AUC_{(0-\text{last})}$ (µg·h/mL)</td>
<td>4.06 ± 1.62</td>
<td>0.98 ± 0.44*</td>
<td>0.25 ± 0.11</td>
</tr>
<tr>
<td>$AUC_{(0-\infty)}$ (µg·h/mL)</td>
<td>4.20 ± 1.66</td>
<td>1.22 ± 0.39</td>
<td>0.30 ± 0.14</td>
</tr>
<tr>
<td>$V_z$ (L/kg)</td>
<td>4.80 ± 0.91</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>$\lambda_{z}$ (h$^{-1}$)</td>
<td>0.55 ± 0.16</td>
<td>0.25 ± 0.06*</td>
<td>0.77 ± 0.55*</td>
</tr>
<tr>
<td>$Cl_1$ (L/h·kg)</td>
<td>2.64 ± 0.84</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>$t_{\frac{1}{2}}$ (h)</td>
<td>1.35 ± 0.40</td>
<td>2.92 ± 0.79*</td>
<td>1.53 ± 1.28*</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.50 ± 0.47</td>
<td>5.10 ± 1.12*</td>
<td>2.56 ± 1.77*</td>
</tr>
<tr>
<td>Disease</td>
<td>No. of Patients</td>
<td>Total Dose</td>
<td>Result good</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gm.</td>
<td></td>
</tr>
<tr>
<td>Pneumonia (16 cases):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumococcal, bacteremic</td>
<td>4</td>
<td>1.4-14.0</td>
<td>2</td>
</tr>
<tr>
<td>Pneumococcal, nonbacteremic</td>
<td>11</td>
<td>2.8-16.8</td>
<td>11</td>
</tr>
<tr>
<td>Etiology not determined</td>
<td>1</td>
<td>3.0</td>
<td>1</td>
</tr>
<tr>
<td>Streptococcal infections (13 cases):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>2</td>
<td>3.0-5.5</td>
<td>2</td>
</tr>
<tr>
<td>Acute follicular tonsillitis</td>
<td>4</td>
<td>2.5-12.6</td>
<td>4</td>
</tr>
<tr>
<td>Scarlet fever</td>
<td>7</td>
<td>7.2-13.5</td>
<td>4</td>
</tr>
<tr>
<td>Staphylococcal infections:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>1</td>
<td>6.0</td>
<td>1</td>
</tr>
<tr>
<td>Enteritis</td>
<td>1</td>
<td>3.4</td>
<td>1</td>
</tr>
<tr>
<td>Diphtheria (carriers)</td>
<td>3</td>
<td>14.0-17.0</td>
<td>3</td>
</tr>
<tr>
<td>Gonorrheal urethritis</td>
<td>4</td>
<td>1.0-3.0</td>
<td>2</td>
</tr>
<tr>
<td>Nonspecific urethritis</td>
<td>3</td>
<td>2.4-5.0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>41</td>
<td></td>
<td>31</td>
</tr>
</tbody>
</table>
# Effects in Pneumonia

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day of Disease</strong></td>
<td>1 2 3 4 5 6 7 8</td>
<td>2 3 4 5 6 7 8 9</td>
<td>1 2 3 4 5 6 7 8 9 10 11</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td><img src="#" alt="Graph A" /></td>
<td><img src="#" alt="Graph B" /></td>
<td><img src="#" alt="Graph C" /></td>
</tr>
<tr>
<td><strong>Erythromycin</strong></td>
<td>200 mg.</td>
<td>200 mg.</td>
<td>200 mg.</td>
</tr>
<tr>
<td><strong>Acute Symptoms</strong></td>
<td>### + 0 0 0 0</td>
<td>### + 0 0 0 0</td>
<td>### + 0 0 0 0</td>
</tr>
<tr>
<td><strong>W.B.C. x 1000</strong></td>
<td>20. 7.6 4.2</td>
<td>20. 17.4 10.8 6.4</td>
<td>13.4 10.9 9.7 7.2 6.6</td>
</tr>
<tr>
<td><strong>Blood Culture</strong></td>
<td>0 0</td>
<td>0 0</td>
<td>Pn.2 0 0</td>
</tr>
<tr>
<td><strong>Sputum Culture</strong></td>
<td>Pneumo.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lungs</strong></td>
<td><img src="#" alt="Lung Diagram A" /></td>
<td><img src="#" alt="Lung Diagram B" /></td>
<td><img src="#" alt="Lung Diagram C" /></td>
</tr>
</tbody>
</table>

*Clear on physical exam*
**Effects in Streptococcal Infections**

<table>
<thead>
<tr>
<th>Cultures for Hemolytic Streptococci</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood cultures positive before treatment</td>
<td>0</td>
</tr>
<tr>
<td>Throat cultures positive before treatment</td>
<td>13</td>
</tr>
<tr>
<td>Repeated throat cultures negative after:</td>
<td></td>
</tr>
<tr>
<td>1 day of treatment</td>
<td>3</td>
</tr>
<tr>
<td>2 days of treatment</td>
<td>4</td>
</tr>
<tr>
<td>6 days of treatment</td>
<td>1</td>
</tr>
<tr>
<td>8 days of treatment</td>
<td>1</td>
</tr>
<tr>
<td>15 days of treatment</td>
<td>1</td>
</tr>
<tr>
<td>16 days of treatment</td>
<td>1</td>
</tr>
<tr>
<td>18 days of treatment</td>
<td>1</td>
</tr>
<tr>
<td>Throat culture positive after 23 days of treatment</td>
<td>1</td>
</tr>
</tbody>
</table>
Post-Market Surveillance

- Reports of infantile hypertrophic pyloric stenosis (IHPS)
  - Narrowing of pylorus in infants associated with projectile vomiting
- 1999 cohort study of 157 newborns treated with erythromycin for pertussis prophylaxis; 7 (5%) developed symptoms and were treated with pyloromyotomy for IHPS
- Proposed a dose-dependent effect:
  - Concluded 5.1% risk of IHPS for erythromycin tx for 8-14 days
  - 10% risk of IHPS for erythromycin tx for 15-21 days
- Erythromycin still in use, but IHPS risk must be considered
Adverse Reactions

- Motilin Agonist - Increases Gastric Emptying
  - Most common: Abd Pain, Nausea, Vomiting, Diarrhea
  - Deters use as first-line antibiotic
- Less common
  - Arrhythmia, Prolonged QT intervals, Torsades de pointes, Deafness, Psychotic reactions, Nightmares, Night sweats
  - Allergies
    - Urticaria, Anaphylaxis, Cholestasis, Stevens-Johnson syndrome, and toxic epidermal necrolysis
- Subpopulation Specific
  - Infants: pyloric stenosis
  - Pregnant: Category B (No teratogenicity or other AR in rats)
Adverse Reactions

- Interactions
  - Combined Oral Contraceptive Pill (COCP)
    - Some clinical evidence of antibiotics lowering plasma contraceptive levels
    - Many proposed mechanisms (altered gut flora)
  - CYP3A4 Metabolizers
    - Warfarin, Statins, Ergotamine
    - Large cohort study showed coadministration of erythromycin with verapamil or diltiazem led to ventricular tachycardia and sudden cardiac death
  - QT Interval Prolongers
    - Terfenadine, Astemizole, Cisapride, Pimozide
Conclusion

- Continuing to see growing antibiotic resistance in common pathogens
- Erythromycin resistant Streptococcus pneumoniae and Strep. pyogenes prompted development of ketolide antibiotics
  - Effective against resistant strains
  - Semi-synthetic derivative of Erythromycin A
- 65 years after release, Erythromycin is a WHO essential medicine
- Remains off first-line antibiotic list due to common gastrointestinal adverse reactions
I think I need antibiotics for my col...

IT'S A VIRUS!