Using Synergy to Potentiate Antibiotics and to Study Mechanisms of Antibiotic Action

Jeffrey H. Miller Lab
UCLA
Staphylococcus aureus
Mycobacterium tuberculosis
Acinetobacter baumannii
Pseudomonas aeruginosa
Brazilian amputee model dead at 20

• STORY HIGHLIGHTS
  • NEW: Brazilian amputee model Mariana Bridi da Costa died early Saturday
  • Da Costa's hands, feet were amputated after she contracted septicemia
  • Da Costa placed sixth in the Miss Bikini International competition in China
“It is time to close the book on infectious disease"

William H. Stewart, U.S. Surgeon General
Testimony before the U.S. Congress, 1969
Resistance to Antibiotics Develops Rapidly

- Sulfonamides
- Penicillin G
- Streptomycin
- Tetracycline
- Erythromycin
- Nalidixic acid
- Methicillin
- Expanded-spectrum cephalosporins
- Augmentin
- Norfloxacin
- Vancomycin
- Oxazolidinone

Some Statistics

- More than 70 percent of the bacteria that cause hospital-acquired infections are resistant to at least one of the antibiotics most commonly used to treat them.

- Many infectious diseases are increasingly difficult to treat.

- Over 2 million fall prey to microbes once they get in the hospital, in this country alone. Some 90,000 die. About 70 percent of those are infected by drug-resistant bacteria. Costs for treatment of these infections approach $5 billion a year. Overall, the yearly toll exacted by drug-resistant infections in the United States is estimated to exceed $30 billion.
Between 1962 and 2000, no major classes of antibiotics were introduced.
Gram-Negative Bacteria

- Multi-drug resistant bacteria are now a serious threat
  - Hospital-acquired *Klebsiella pneumoniae*
  - In a 2011 outbreak at the U.S. National Institutes of Health Clinical Center, 11 of 18 affected patients died (Snitkin et al. 2012)
Approaches/Methods

- Find new antibiotics from natural sources
- Make chemical derivatives of existing antibiotics
- Find new targets in cell
- Find new combinations of existing drugs
- Find co-drug targets and co-drugs (potentiators)
Recent Approaches

1. Finding natural products derived from nonconventional sources (plants, marine microorganisms, insects)

2. Using rational design to make new chemical derivatives of existing antibiotics from 3D structures

3. Linking peptides to drugs to facilitate entry through cell membranes
Our Approach

Investigate synergy with new combinations of already approved antibiotics
Thank you to...

Tina Kang
Jessica Yuan
Alice Zhou
Casey Beppler

Caroline Nguyen
Jack Mao
Minh Quan Nguyen
Chris Vu
Thank you to...

Lisa Song  Pamela Yeh
Pairwise Interactions of Antibiotics

- Pamela Yeh, et al. 2006

- Classified drugs according to similar interactions with other antibiotics

- Groups formed according to biochemical function
Pairwise Interactions of Antibiotics

- General Procedure:
  1) Define sub-lethal concentrations for each antibiotic
     Concentration that doesn’t kill, but leads to about 50-90% growth
  2) Test no-drug control, drugs individually, and drugs in combination
  3) Define interaction as synergistic, additive, antagonistic or suppressive
Questions

1. Can we expand the drug interaction network?
2. Can we use synergy to potentiate antibiotics?
3. What is the mechanism of synergy?
4. How do bactericidal antibiotics kill cells?
5. Can we use synergy to test models of killing?
a. Triclosan

b. Aztreonam

c. Rifampicin
Vancomycin
Why vancomycin?

- Highly potent drug
- Mechanism of action: Inhibits cell wall synthesis in Gram-positive bacteria
But...

- Extremely large molecule
- Unable to penetrate the outer membrane of Gram-negative bacteria
Why use *Escherichia coli*?

- Avoid working with pathogenic *K. pneumoniae*
- Also a Gram-negative bacteria
  - Specifically gammaproteobacteria
  - Behaves similarly to *K. pneumoniae*
    - Enzymes
    - Metabolism
    - Permeability
    - Drug interactions
Can we find a way to use vancomycin against Gram-negative bacteria?

- Clinically relevant doses in wild-type *E. coli* are ineffective
- Highly synergistic interactions with vancomycin could potentiate its effects
- Study drug interactions to develop combinatorial drug therapies
Vancomycin (μg/ml) susceptibility of WT in LB medium
Pairwise Interactions of Antibiotics

- Yeh, et al. did not include vancomycin.
- The inefficiency of vancomycin in wild-type *E. coli* makes it a poor candidate for initial studies of drug interactions.
- Instead, we can take advantage of weakened backgrounds to see how vancomycin might interact with other drugs in *E. coli*.
  - SurA-deficient
SurA-Deficient *E. coli*

- peptidyl-prolyl cis-trans isomerase (SurA)
- Periplasmic molecular chaperone that facilitates the folding and assemblage of outer-membrane proteins in Gram-negative bacteria
- SurA-deficient *E. coli* more sensitive to certain antibiotics
Vancomycin MIC (μg/ml)

- WT: 500
- surA: 4
- smpA: 70
- surA smpA: 1.5
Experimental Design

1) Define sub-lethal dose for each background.

2) Test for synergy in the SurA-deficient background.

3) Test promising synergistic combinations in the wild-type background.
## Drugs and Mechanism of Action

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Results: SurA-deficient background

![Graph showing percent growth against drug concentration (μg/ml) with control and combination with VAN]
Vancomycin interactions with other drug classes in surA background
Wild-type background

Drug Concentration (µg/ml)

Percent Growth

Percent Growth

LB    VAN 25    NTR 3.5    VAN 25 + NTR 3.5

LB    VAN 12.5    NTR 3.5    VAN 12.5 + NTR 3.5
Wild-type background

Drug Concentration (μg/ml)

Percent Growth

Drug Concentration (μg/ml)
Questions

- How do bactericidal antibiotics kill cells?
- Do each have a unique mechanism based on their initial target?
- Do they all kill via a common mechanism?
- Are there multiple mechanisms operating at the same time?
A Common Mechanism of Cellular Death Induced by Bactericidal Antibiotics

Michael A. Kohanski,1,2,5,6 Daniel J. Dwyer,1,3,6 Boris Hayete,1,4 Carolyn A. Lawrence,1,2 and James J. Collins1,2,3,4,*

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DOI 10.1016/j.cell.2007.06.049
Oxidative damage killing pathway

- Hydroxyl radicals lead to damaged DNA bases and double strand breaks
- The RecABC system repairs the breaks
- When the damage exceeds the repair capacity, cell death results
Hypothesis

- Synergy between bactericidal drugs can result from each drug contributing damage that results in double strand breaks.
- Can test this by using known compounds that cause double strand breaks via known mechanisms, and thus act as antimicrobials.
- These compounds include commonly used mutagens.
4-Nitroquinoline 1-oxide (4NQO) oxidizes guanines in the DNA, leading to double strand breaks.

4NQO should therefore show strong synergies with bactericidal antibiotics, but not bacteriostatic drugs.
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Other Mutagens

- Zebularine
- 5-Azacytidine
- 2-Aminopurine
- 5-Bromodeoxyuridine
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The table represents various reactions or interactions, likely in a scientific context. The colors and notations suggest different types of reactions or conditions.
Conclusions

- Vancomycin exhibits strong synergistic interactions with at least nitrofurantoin and trimethoprim.
- Synergy experiments validate the oxidative damage route to cell killing.
- Synergy can be explained in many cases as a dose effect of double strand breaks, occurring in later step (ultimate) targets.
Future Directions

- Mutagenic Fingerprints of antibiotics