Penicillin G and Penicillin Binding Protein 4: A Patient Case Related to Medicinal Chemistry and Drug Design

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Abstract

Penicillin G (Figure 1) can enhance the effect of warfarin indirectly, leading to possible bleeding problems. Penicillin G binds to and destroys bacteria in the intestines that produce vitamin K. Vitamin K is used in our body to create clots in bleeding episodes. Warfarin is used as a blood thinner and makes our blood less likely to clot. When penicillin G and warfarin are used in combination, a dangerous decrease in blood clotting occurs, making for an increased risk for life threatening bleeds. The following poster is a review of the indirect drug interaction between penicillin G and warfarin and will discuss a patient case dealing with this drug-drug interaction.

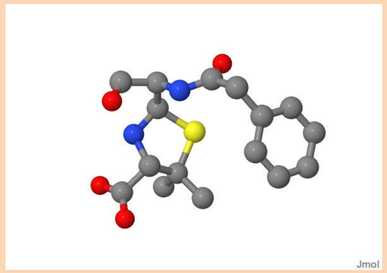

Figure 1: Molecular structure of penicillin rendered from 2EX8.pdb.

Penicillin molecules: gray = carbon; red = oxygen; blue = nitrogen; yellow = sulfur.

Introduction

A 26-year-old male was admitted to St. Vincent Hospital complaining of a cough, fever, and general fatigue. An echocardiogram and lab test showed the patient had infective endocarditis caused by *Staphylococcus aureus*. The man had been admitted to the hospital on multiple occasions for poor health associated with IV drug use, which was believed to be the source of the infection as well. If a person fails to use sterile technique when using a needle to inject themselves, the bacteria on the skin goes straight into the bloodstream and to the right side of the heart.[1] The physician ordered penicillin G potassium, 4 million units IV every 4 hours. Penicillin kills *S. aureus* among other bacteria, such as *Escherichia coli* and *Bacteroides fragilis*. The pharmacist noticed the patient was also taking warfarin (an oral anticoagulant) for a previous condition and knew that penicillin G can enhance the effect of warfarin. However, the antibiotic was needed to save the man's life, so the medication order was approved, along with a note to closely monitor the patient's International Normalized Ratio (INR) due to the indirect drug-drug interaction. INR is a standardized measure of how long it takes a patient's plasma to clot.

Infective endocarditis is an infection and inflammation of the heart. While many different microorganisms can cause endocarditis, the most common cause is bacterial, specifically members of the *Staphylococcus* genus. Endocarditis can lead to several complications, the most dangerous and lethal of which is congestive heart failure. All cases need to be treated as quickly as possible with an antimicrobial agent that the microbe is susceptible to.[2] One option for antimicrobial therapy is penicillin G, which binds to penicillin binding proteins and will be discussed in more detail.

Molecular Story

Penicillin Binding Protein 4 (PBP4) Structure:

- Consists of three domains (Figure 2)[3]
  - Domain I consists of residues 1-80 and 294-477
  - Domain II consists of residues 81-172 and 248-292
  - Domain III consists of residues 173-247
- Enzymatic activity is found in Domain I[4]
  - N-terminus contains transpeptidase activity (Figure 3)
    - Consists of five-stranded antiparallel β-sheet between two helical clusters
      - One cluster has seven helices and the other has two helices
    - Active serine residue (serine-X-X-lysine) located in the larger α-helix cluster
  - C-terminus contains carboxypeptidase activity (Figure 3)
    - Consists of two antiparallel β-sheets
    - Contains conserved sequences
      - serine-X-asparagine
      - lysine-threonine-glycine
    - Binding site for penicillin
- Penicillin binding site (Figures 4 and 5)[3]
  - Pocket consists of three main residues, each in the context of a conserved sequence
    - Ser-X-X-Lys → catalytic residue
      - PBP4: Pro-Ala-**Ser62-Thr-Gln-Lys**-Val-Ile-Thr-Ala-Leu
    - Ser-X-Asn → Beta-lactam antibiotic target
      - PBP4: Lys-Ile-Met-Leu-Lys-Lys-**Ser306-Asp-Asn**-Met-Ile
    - Lys-Thr-Gly → Beta-lactam antibiotic target
      - PBP4: Val-Asp-Gly-Lys-Val-Ser-Ala-**Lys-Thr418-Gly**-Ser

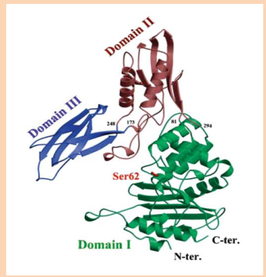

Figure 2: 3D ribbon model of PBP4 from *E. coli* showing its 3 domains and the active serine residue.[3]

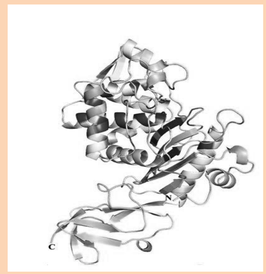

Figure 3: 3D ribbon model of PBP4 domain I showing the N-terminal and C-terminal ends.[4]

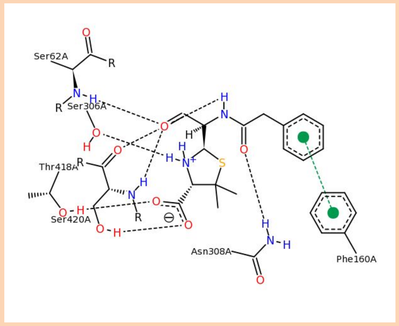

Figure 4: 2D diagram of the active site residues of PBP4 and their interactions with penicillin.[3]

Key residues are Ser62, Ser306, and Thr418. Phe160 is located in domain II.

Penicillin G

- Beta-lactam antibiotic
- Inhibits bacterial wall synthesis in gram positive and gram negative organisms
- Forms an irreversible covalent bond with PBP4 at the C-terminal end initiated by acylation involving a nucleophilic attack by serine[5]
  - Permanently inactivates PBP4, which inhibits its functions associated with cell wall formation[5]
  - Cell wall is weakened to the point of autolysis →bacterial cell death → fewer microbes and less vitamin K synthesis

Warfarin

- Action relies heavily on stable levels of vitamin K
- Antagonizes vitamin K epoxide reductase → inhibits the synthesis of clotting factors II, VII, IX, and X
  - Vitamin K reduction → toxic levels of warfarin → spikes in INR values → increased risk of bleeding

The medical staff for the patient presented in the case received orders from the pharmacist for strict monitoring due to the indirect interaction between penicillin G and warfarin and its effects on bleeding risks.

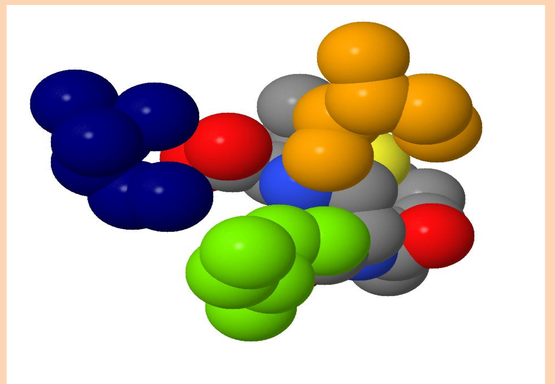

Figure 5: Three main PBP4 residues interacting with penicillin rendered from 2EX8.

Penicillin molecules: gray = carbon; red = oxygen; blue = nitrogen; yellow = sulfur.

PBP4 molecules: green = Ser62; orange = Ser306; navy blue = Thr418.

Future Work

The indirect interaction between warfarin and penicillin G is due to penicillin binding to and killing microbes, decreasing the synthesis of vitamin K. The vitamin K reduction leads to warfarin toxicity and increases the risk of bleeding. Penicillin G could be modified to improve selectivity to a specific microbe, such as *S. aureus*. Through increasing specificity to *S. aureus*, other normal flora, such as *E. coli and B. fragilis*, would not interact with penicillin. Thus, normal flora would not be killed and vitamin K and clotting factors would still be produced without decreasing the effectiveness of the antibiotic. This would lead to less warfarin toxicity and less bleeding risks when taking penicillin.

Enzyme	Microorganism	Active Site Sequence							
CPase	*Bacillus subtilis*	-Leu	-Pro	-Ile	-Ala	-Ser	-Met	-Thr	-Lys-
CPase	*Bacillus stearothermophilus*	-Leu	-Gly	-Ile	-Ala	-Ser	-Met	-Thr	-Lys-
PBP 5, 5[1]	*Escherichia coli*	-Arg	-Asp	-Pro	-Ala	-Ser	-Leu	-Thr	-Lys-
PBP 1b	*Escherichia coli*		-Ser	-Ile	-Gly	-Ser	-Leu	-Ala	-Lys-
PBP 3	*Escherichia coli*	-Phe	-Glu	-Pro	-Gly	-Ser	-Thr	-Val	-Lys-
PBP4	***Escherichia coli***	**-Ala**	**-Leu**	**-Pro**	**-Ala**	**-Ser**	**-Thr**	**-Gln**	**-Lys-**
Class A	*Staphylococcus aureus*	-Phe	-Ala	-Tyr	-Ala	-Ser	-Thr	-Ser	-Lys-
β-lactamase	*Bacillus cereus*	-Phe	-Ala	-Phe	-Ala	-Ser	-Thr	-Tyr	-Lys-
	Bacillus licheniformis	-Phe	-Ala	-Phe	-Ala	-Ser	-Thr	-Ile	-Lys-
	Escherichia coli	-Phe	-Pro	-Met	-Met	-Ser	-Thr	-Phe	-Lys-
Class C	*Escherichia coli*	-Phe	-Glu	-Leu	-Gly	-Ser	-Val	-Ser	-Lys-
β-lactamase	*Pseudomonas aeruginosa*	-Phe	-Glu	-Ile	-Gly	-Ser	-Val	-Ser	-Lys-

Table 1: Amino acid sequence of the penicillin binding protein active site.[5]

Active serine residues (serine-X-X-lysine) are highlighted in red. Amino acid unique to *S. aureus* in sequence is highlighted in blue. Amino acids unique to *E. coli* in sequence are highlighted in green.

As stated earlier, the serine-X-X-lysine section on the penicillin binding protein is vital to the function of penicillin. This active serine is present on various bacteria (Table 1). To increase penicillin selectivity to a specific microbe, penicillin would need to be modified to bind to an additional site on the protein that is more specific to the particular bacteria. For example, the active site sequences for *S. aureus* and *E. coli* are very similar, as seen in Table 1. However, there is a tyrosine amino acid present in the *S. aureus* sequence that is not present in the *E. coli* sequence. Penicillin could be altered to bind to the tyrosine portion in addition to the active serine section to improve selectivity to *S. aureus* and to decrease indirect interactions with warfarin.

Summary

As previously discussed, penicillin covalently binds to PBP4 receptors within various microbes, including *S. aureus* and *E. coli*, which leads to weakened cell walls and, ultimately, autolysis. The penicillin kills the bacteria that produce vitamin K. Since there is less vitamin K present after taking penicillin, there is an increased risk of bleeding when concurrently taking warfarin. Modification of the penicillin molecular structure is the next step to producing a drug that binds more specifically with the microorganism resulting in fewer drug interactions.

References

1. Ashley, et al. (2004) *Cardiology Explained*, 167.
2. Black, et al. (2005) *Microbiology: Principles and Explorations*, 686.
3. PDB ID: 2EX8. Kishida, et al. (2006) *J. Biochem.,* 45: 783.
4. Navratna, et al. (2010) *J. Bacteriol.*, 192(1): 134.
5. Hirota, et al. (1985) *Bacteriol.*, 164(1): 456.

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