Ertapenem: A Patient Case Related to Medicinal Chemistry and Drug Design

Poster Team: Jolene Dutcher, Phoua Xiong, Tyron Gruber, Victoria Strong, Benjamin Kluge
Jmol Team: Joshua Lee, Craig Irwin, Julie Schears
Faculty Advisors: Daniel Sem, Ph.D. and Douglas Borys, Pharm.D., DABAT
Professional Mentor: Phil Ollinger, Pharm.D., Froedtert Hospital, Milwaukee, WI

Concordia University Wisconsin School of Pharmacy, 12800 N Lake Shore Drive, Mequon, WI, 53097

Abstract

Escherichia coli is an example of a carbepenem-resistant Enterobactericieae (CRE). This family of bacteria is becoming difficult to treat due to its resistance to broader antibiotics such as ertapenem, a member of the carbapenems.[1] *E. coli*, being a gram negative species and a part of the normal human flora, has the potential to cause serious infection due to the expression of novel resistant pathways. Broad spectrum antibiotics like carbapenems pose the risk of lowering our human microbial flora, allowing invasive bacterial strains to colonize; one prominent species being *E. coli.* Of these pathways, studies have shown increasing rates of mutation in key residues in the OmpF membrane protein, a porin that allows substrates to enter from the extracellular space into the periplasmic space. Mutations in these porins prevents the entry of carbapenem antibiotics.[2] Although these cases are rare, CREs are very dangerous and incidents are increasing. Additionally, with E. coli's ability to conjugate with other gram negative species, it is important to track resistance patterns throughout the United States.

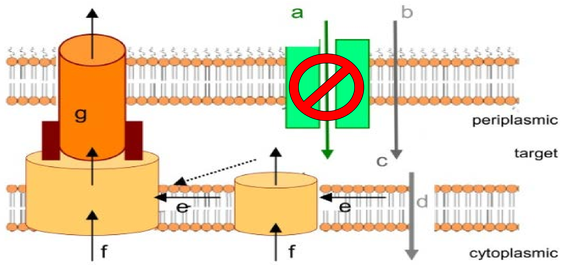

Figure 1: Typical mechanism of antibiotic entrance into a gram-negative bacteria species. a) active transport of hydrophilic substrate through the OmpF porin protein, b) passive transport of hydrophobic substrate, c) substrate in periplasmic space, d) transport of substrate into intracellular space, e) active transport of the substrate to the efflux pump complex f and g. The cancel sign over OmpF is to show the mechanism of a mutation in transport of substrates, essentially blocking movement of substrate into the periplasmic space of the bacteria.[3]

Introduction

Patient Case

A 35 year old male patient was admitted to Froedtert Hospital several times in the past few months for a chronic bacterial infection and had been prescribed several antimicrobial agents. On this visit, he had a temperature of 103 degrees Fahrenheit and severe diarrhea. Labs showed he was positive for *E. coli* induced enteritis. The doctor ordered ertapenem 1g over 30 minutes IV infusion once daily. After five days of treatment with no improvement, the physician concluded that the patient had a CRE and questioned if ertapenem is an appropriate treatment choice.

Ertapenem

Ertapenem is an intravenously available carbapenem used to treat Extended Spectrum Beta-Lactamase (ESBL) gram negative species such as *Klebsiella* and *Escherichia*.[4] The mechanism of action is the same as beta-lactam antibiotics: inhibit penicillin binding proteins (specifically the Transpeptidase enzyme as seen in Figure 6) and block the synthesis of the bacterial cell wall.[5] In the absence of the cell wall, water will move into the cell and cause the pressure of the cell to increase to the point of lysis. Due to the structure of carbapenems, they are resistant to many beta-lactamases, which are produced by bacteria to inhibit antibiotics with a beta-lactam core; this makes them broad spectrum agents for gram negative species.[5, 6] The normal mechanism of entry for carbapenems is highlighted in Figure 1.

Molecular Story

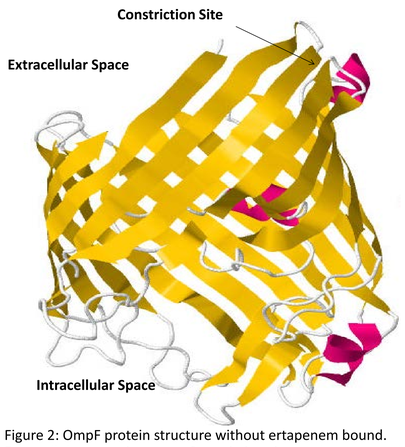

Figure 2: OmpF protein structure without ertapenem bound. Extracellular space is on top. Constriction site can be seen on the top right side where the alpha helix lies extracellularly. Yellow arrows represent beta sheets , pink spirals are alpha helixes, and white strands are loop domains.

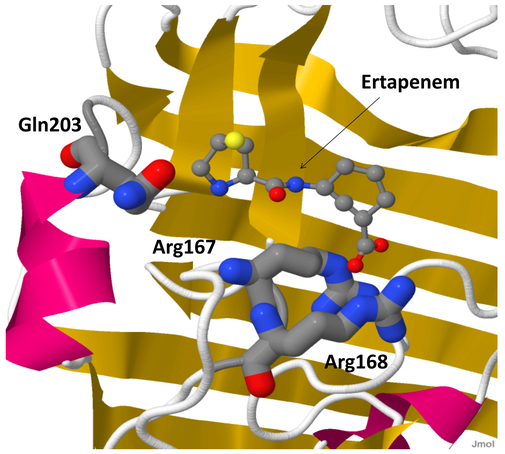

Figure 3: Ertapenem hydrogen binding to Gln203, Arg167, & Arg168 of the extracellular loops of the OmpF porin of *E. coli*. Yellow arrows represent beta sheets , pink spirals are alpha helixes, and white strands are loop domains.

Ertapenem Binding to OmpF

The beta strands of the OmpF porin on the periplasmic side of the bacterial cell membrane are connected through beta-turn motifs, while the extracellular side has loops that perform various functions (stabilization of the trimer structure as well as the formation of a "constriction zone" which regulates what size molecules can transverse the pore).[5] Through crystallization, it was revealed that ertapenem is bound to the OmpF porin in an orientation parallel to the porin near the extracellular loops (Figure 3). Ertapenem is bound to the extracellular loops through hydrogen bonding (within 3.3 angstroms) of 3 residues: Gln203, Arg167, & Arg168 as shown in Figure 4.[5]

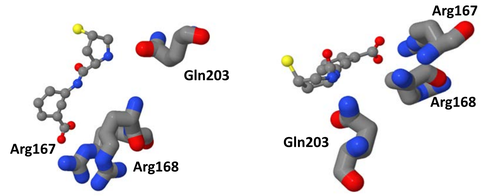

Figure 4: Close up of Ertapenem hydrogen binding to Gln203, Arg167, & Arg168 of the extracellular loops of the OmpF porin of *E. coli* in two different orientations.

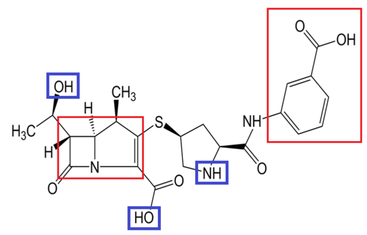

Figure 5 (Above): Structure of ertapenem. Red box on the left indicates the carbapenem ring present in all carbapenems. The red box on the right shows the side chain that confers resistances to extended spectrum beta-lactamases, giving ertapenem its broad spectrum activity. Blue boxes show possible ionization states at physiological pH (7.3).

Like many porin proteins, OmpF is comprised of three independent monomer β-barrels that allow larger charged molecules to enter the periplasmic space of the gram-negative bacteria.[7] Ertapenem, a larger, hydrophilic molecule at physiological pH, has two negatively charged carboxylate groups and one positive amine group as shown in Figure 5. It is also attracted to the constriction site on the extracellular surface of OmpF shown in Figure 3. This may be due to the constriction site's loop domain having a highly concentrated negative charge because of the acidic residues. Another reason this attraction may occur is because the face of the β-barrel has positively charged and basic amino acids. The interaction within the gap forms an electric field that attracts ertapenem's charged groups and allows reversible binding.[5] When in contact with the β-barrel, the negatively charged carboxylate on ertapenem orientates itself parallel with the positively charged β-barrel and basic amine of ertapenem. This conformational change causes the carboxylate groups to face towards the constriction site in the extracellular loops.

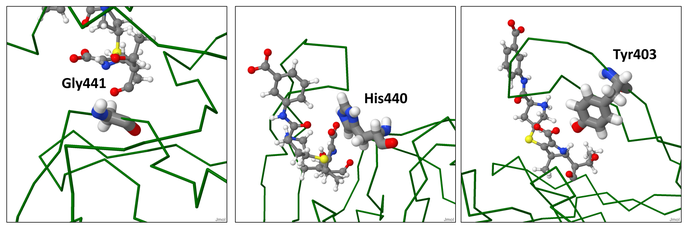

Figure 6 (Right): Hydrogen bonding interactions between ertapenem and Tyr403, His440 and Gly441 of *Enterococcus faecium* L,D-Transpeptidase; one target of beta-lactam based antibiotics. The green wire represents the protein amino acid tertiary structure.[8]

Future Research

Overcoming Resistance

The greatest difficulty with this antibiotic is overcoming the bacterial porin resistance. Ertapenem is bound to the OmpF porin near the extracellular loops of the pore via hydrogen bonding of 3 OmpF residues (Gln203, Arg167, and Arg168). This occurs due to the negatively charged carboxylic acid residue at the bottom of the benzene ring. To reduce the hydrogen bonding, a less polar or more lipophilic functional group could be inserted. Additionally, resistance at the OmpF porin also occurs due to the orientation that the molecule takes when bound to the porin (ertapenem's arrangement is parallel to the pore axis). Binding orientation of the drug could be influenced by rearranging and shortening the side chains (particularly the side chain opposite of the carbepenem ring) or by introducing an amine connector within the molecule that functions similar to that found in ampicillin and carbenecillin.

Ertapenem itself also exists in a charged state at physiological pH, needing active or facilitated transport through the membrane. Substituting the amine and carboxylic groups with bioisosteres that aren't charged at pH 7.3 may override the need for porin assistance to get into the periplasmic space. This would make the drug more lipophilic, consequently increasing ertapenem's absorption, plasma protein binding, volume of distribution, and clearance.

Summary

Patient Profile

After five days of dosing (1g intravenously over 30 minutes once daily), the patient's symptoms of diarrhea and fever remain unchanged and he was declared to have CRE. Per policy and protocol of Froedtert Hospital, the physician dosed the patient with another antibiotic that would be more effective against CRE such as fosfomycin or tigecyline.

Resistance to Ertapenem

The carbapenem antibiotic resistance presented in the case is caused by a porin protein (specifically OmpF) mutation, resulting in different amino acid expression. The change in residues causes the porin to prevent molecules like carbapenems from entering through the outer membrane . Molecules may also bind to the porin protein itself, signaling the molecule for efflux. Regardless of the mechanisms listed, the antibiotic is unable to penetrate the outer membrane of *E. coli*.

References

Paper Resources

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7. Kojima et al. (2013). *Proceedings of the National Academy of Science USA*, 10(28), 2629.
8. Lecoq et al. (2013). *ACS Chemical Biology*, 1140-1146.

Images

- Figure 1 - Masi et al. (2013). *Open Microbiology*, 22-33. Image rendered by Benjamin Kluge using Paint.
- Figure 2, 3, 4 - Zievogel (2012). *Structure*, 8(21), 76-87. 4GCS.PDB. Images rendered by Joshua Lee using Jmol.
- Figure 5 - Retrieved from http://dailymed.nlm.nih.gov/. Image rendered by Benjamin Kluge using Paint.
- Figure 6 - Lecoq et al. (2013). *ACS Chemical Biology*, 1140-1146. 3ZGP.PDB. Images rendered by Joshua Lee using Jmol.

The CREST Program is funded by NSF-DUE grants #1022793 and #1323414.