The Mechanism of Penicillin and Emergence of Resistance

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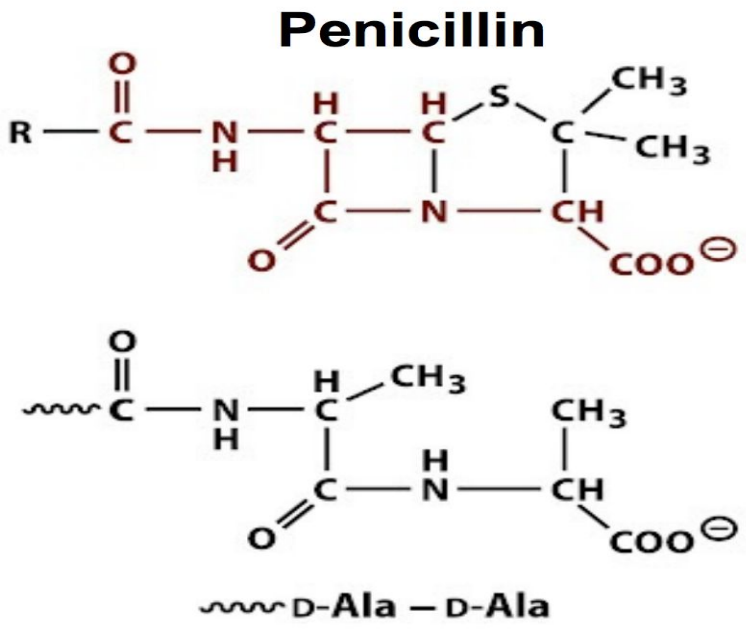
Introduction

Before WWII, bacterial infections would most likely lead to death. In 1928, Alexander Fleming, a British biologist, accidentally discovered penicillin after observing the disappearance of bacterial colonies in the presence of mold. After the isolation of penicillin, the number of deaths as a result of bacterial infections drastically decreased. Penicillin is a member of the β-lactamase family of antibiotics and works to inhibit cell wall biosynthesis in bacteria, leading to cell death. This process is achieved through two steps; the first being binding to the target protein resulting in the inhibition of transpeptidase, a bacterial enzyme which cross-links peptidoglycan for cell wall biosynthesis. The second step is the continual functionality of the bacterial enzymes that break down the cell wall for remodeling which results in cell wall degradation as the biosynthetic step is no longer occurring. However, bacteria have been shown to become resistant to antibiotics such as penicillin, greatly reducing their effectiveness by the following two steps. The first step being acquiring a genetic mutation that inhibits the binding of penicillin. The second through spreading the beneficial mutation to their progeny along with other cells.

The purpose of this document is to inform the reader about the mechanism in which penicillin kills bacterial cells in addition to how bacteria can counteract the antibiotic. The reader is expected to be familiar with most of the terminology from a cellular biology class but is likely unfamiliar with the mechanism of penicillin. The author will attempt to explain the terminology for those who are not as familiar.

Figure 1.



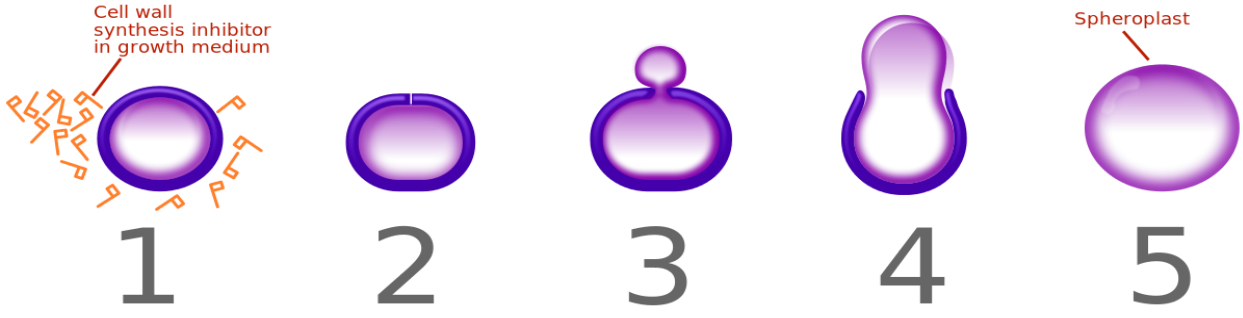
The structure of Penicillin in comparison to D-alanine-alanine.
Image from Dr. Manuel of Micrb 412

Binding to the cell & inhibition of transpeptidase

Before penicillin is able to begin its destruction of the cell, it must bind to the enzyme DD-transpeptidase (peptidoglycan building) by mimicking the shape of D-alanine-alanine. D-alanine-alanine and penicillin are both four membered rings and penicillin functions to mimic the structure of D-alanine-alanine. In addition to both being four membered ring structures, penicillin has oxygen and nitrogen atoms in similar positions to D-alanine-alanine, as well as containing similar functional groups such as an amide (nitrogen containing) and a carboxylate ion (a carbon bonded to two oxygens). These structures can be observed in Figure 1 for direct comparison. This similar structure allows penicillin to trick the enzyme DD-transpeptidase into binding to penicillin instead of D-alanine-alanine.

The process of cell wall formation necessitates the bacteria creating "holes" in its cell wall of peptidoglycan to allow for growth. Penicillin makes it so that when a bacterial cell divides in its presence, the cell cannot fill in the "holes" it creates. Once the binding of penicillin takes place, the DD-transpeptidase enzyme is no longer able to catalyze the formation of the cross-links (attaching one peptidoglycan to another) that typically occur within peptidoglycan in the cell wall. Peptidoglycan is a polymer of sugars and amino acids that makes up most of the cell wall. The cross-linking can no longer occur because the D-alanine-alanine is unable to form chains with itself as peptidoglycan is able to due to the mild differences in its chemical structure. Thus, the DD-transpeptidase enzyme is inhibited as it no longer has a substrate to cross-link in order to build peptidoglycan. This process results in an imbalance between peptidoglycan production and degradation, eventually leading to cellular death.

Figure 2.



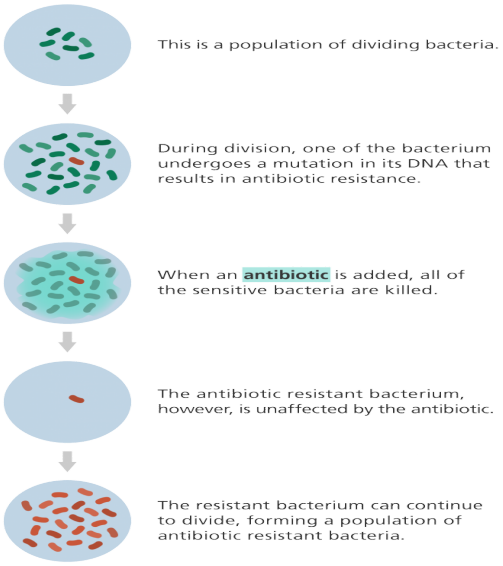
Bacteria that attempt to grow and divide in the presence of penicillin fail to do so, and instead end up shedding their cell walls.
Image from http://www.jbc.org/content/255/9/3977.full.pdf

Cell wall degradation

While the enzymes that build up the peptidoglycan such as DD-transpeptidase are inhibited, the other enzymes that hydrolyze (break down) the peptidoglycan such as peptidoglycan hydrolase continue to function. While the peptidoglycan hydrolase is breaking down the old peptidoglycan to be reformed during cellular growth, the DD-transpeptidase is inhibited and thus unable to reform the peptidoglycan in its place. This results in the weakening of the bacterial cell wall, and consequently an influx of extracellular fluids that disrupt the osmotic (water) pressure of the cell. This process can be seen in Figure 2 as the cell wall is shed as the peptidoglycan is not reformed.

The disruption of the osmotic pressure will eventually cause death through a process known as cytolysis where the cell will burst due to the imbalance. Moreover, the relatively small size of penicillin allows it to penetrate deep into the cell wall, ensuring that the entirety of the cell wall is compromised. The functionality of penicillin does not damage the cells of the patient as animal cells do not contain cell walls.

Figure 3.



A simple description of the development of antibiotic resistance.
Image from http://www.yourgenome.org/facts/what-is-antibiotic-resistance

Antibiotic resistance genetic mutation occurs

Virtually all antibiotics have antibiotic resistant bacteria, including penicillin. During the process of DNA or RNA replication in bacteria, mutations can emerge just as they can in humans. While many mutations are fatal to the bacteria, some will be beneficial and may cause genetic resistance to an antibiotic such as penicillin. These mutations often arise through random point mutations of a genetic sequence where a single or multiple nucleotides (genetic coding molecules) will be altered. This change can result in the alteration of the DD-transpeptidase binding site of penicillin resulting in the bacteria becoming resistant to penicillin. Without the ability to bind to the target enzyme, penicillin is rendered ineffective and the bacteria will continue to inflict disease on the host.

Antibiotic resistance transmission

As shown in Figure 3, only one resistant bacteria is required to accumulate a resistant bacterial population and negatively impact the host they infect. Penicillin will kill all cells that are not resistant, leaving the bacteria that is resistant to penicillin alive which causes the population to shift in favor of penicillin resistance. Bacterial cells initiate a dividing processes similar to animal cytokinesis, resulting in one cell splitting into two separate cells. Through this process and thousands of divisions, a single resistant cell can turn into millions of resistant cells in a short period of time as bacterial cells tend to divide every 20-30 minutes. This results in an

exponential increase of resistant cells that penicillin will no longer be able to inhibit as the genetic resistance is passed on. Furthermore, a single bacterial cell is able to transfer its resistance genes to sensitive cells and even other species through a process known as conjugation. Conjugation occurs when a resistant bacteria gives a sensitive bacteria a resistance genome through an extension of its cell known as a pili. This pili is an elongated protrusion of the membrane that is hollow and allows genetic information to be passed from one cell to another. After the genetic information is transferred, the newly acquired resistance gene is quickly incorporated into the bacterial genome to produce antibiotic resistance effects. The process results in not only individual bacterial resistance, but resistance that is in a bacterial population and that can be spread to other bacterial species, greatly dampening the effectiveness of penicillin.

Conclusion

Penicillin was a revolutionary drug that enhanced life expectancy and made numerous other medical procedures more accessible. Through its inhibition of cell wall biosynthesis, penicillin is able to prevent bacteria from causing severe illness in host organisms. Penicillin inhibition of cell wall biosynthesis begins with binding to the target protein to prevent enzymatic activation, followed by the bacteria cell shedding its own cell wall. However, a bacterial population can become resistant to penicillin by acquiring a resistance gene through genetic mutation and passing it to their progeny and other cells.