Sulfonamides, Antifolates & Quinolones

-Agents that interfere with Nucleic Acid Biosynthesis

- The three major classes of agents discussed here have two features in common:
  a) they are all synthetic compounds; that is, they are not derived from microorganisms
  b) they all selectively interfere, in various manners, with bacterial nucleic acid biosynthesis

I. Sulfonamides - the oldest class of antibiotics

A) Structures

1) History -
   It is appropriate to give a brief description of the history of the sulfonamides, as they were the first antibiotics developed. Scientists at Bayer in Germany discovered in the early 1930’s that protonsil rubrum, a red dye, would block infections in laboratory animals. Surprisingly, this compound did not block infections in vitro. It was discovered later in the decade that liver enzymes were responsible for metabolizing the inactive dye to the active antibacterial agent sulfanilamide, the parent sulfonamide and the first antibiotic (see figure below). Sulfanilamide is no longer used clinically, but it is important not only for its role as a precursor to the clinically used sulfonamides, but also as a precursor to the sulfonamide anti-glaucoma agents.

2) Examples of Clinically Useful Sulfonamides

Sulfanilamide and other older sulfonamides are used either very sparingly or not all clinically today, due in large part to their propensity to crystallize out of urine. The two sulfonamides that are shown below (sulfamethoxazole and sulfisoxazole) do not have this propensity, and thus are the two primarily used sulfonamides. Sulfonamides are usually administered orally, and their primary use is for the treatment of urinary tract infections. They are primarily active against gram-negative bacteria.
3) Related Agents

Two agents closely related to the sulfonamides are dapsone and para-aminosalicyclic acid (see below). Dapsone is used in the treatment of leprosy and para-aminosalicyclic acid is used as a second-line treatment for tuberculosis. Both of these compounds have structural similarities to the sulfonamides, and act at least in part via the same mechanism.

![Chemical structures of Dapsone and Para-aminosalicyclic Acid]

B) Key Structural Features

The key to the mechanism of sulfonamides is their resemblance to para-aminobenzoic acid. Thus, the key structural features of these drugs is a) the presence of a para-aminophenyl moiety and b) the presence of an acidic hydrogen on the sulfonamide that can mimic the acidic hydrogen on the carboxylate of para-aminobenzoic acid.

![Chemical structures of Acidic Hydrogen and Para-aminobenzoic Acid]

C) Mechanism of Action

Sulfonamides exert their antibacterial effect by blocking the production of folates in bacteria. Folates are required for several one-carbon transfer reactions involved in the production of new purine and pyrimidine bases, which are required for the production of new DNA. Thus drugs that block folate metabolism block bacterial cell replication. Folates are required for the biosynthesis of purines and pyrimidines in humans as well. However, the selective toxicity of folates to bacteria is due to a key difference in folate metabolism between bacteria and humans (see figure below). For humans, folic acid is a vitamin that is acquired in the diet. Most bacteria, on the other hand, synthesize their own folic acid. Sulfonamides inhibit an enzyme, dihydropteroate synthase, that is part of the bacterial folate biosynthetic pathway. **We are not affected by sulfonamides because we do not have this enzyme.** Thus, dihydropteroate synthase is an ideal target for an antibacterial agent. However, note that this mechanism of action provides a convenient way for the bacteria to evade the antibacterial effects of sulfonamides - they can simply obtain folates from their environment in the same way that we do. Thus, enterococci are resistant to sulfonamides because they naturally are able to take up folates from their environment. Resistance can also occur through the acquisition of a plasmid-based mutant dihydropteroate synthase that has reduced affinity for sulfonamides.
D) Metabolism and Elimination

Most of a dose of a sulfonamide is excreted unchanged into the urine, which is one of the key reasons that sulfonamides are valuable agents for the treatment of urinary tract infections. For the small portion of the dose that is not excreted in an unchanged manner, one of the important metabolic routes is N-acetylation of the free para-amino group in the liver.

E) Mechanisms of Toxicity

The most common side effect that occurs with sulfonamides is hypersensitivity, which is frequently manifested in the form of rashes. Various serious blood disorders can be seen in patients who are being treated with sulfonamides (acute hemolytic anemia, agranulocytosis, aplastic anemia) but these are VERY rare. Crystallization of sulfonamides into the urine was a problem with sulfanilamide and other sulfonamides that were introduced in the 1930’s and 1940’s, but this is not a problem with sulfamethoxazole and sulfisoxazole, the sulfonamides currently used.
II. Trimethoprim & Related Antifolates

A) Structure
Unlike sulfanilamide, which was discovered through random screening of dye molecules for their antibacterial effects, trimethoprim was developed in the 1950’s through a targeted drug discovery effort to identify compounds that selectively inhibited bacterial dihydrofolate reductase (DHFR). This effort was led by Gertrude Elion and George Hitchings at Burroughs-Wellcome, and for this work they received the Nobel Prize in 1988.

Trimethoprim is primarily used in combination with sulfamethoxazole (vide infra) for the treatment of urinary tract infections, and can be given either orally or by intravenous infusion.

B) Mechanism of Action
Trimethoprim, like the sulfonamides, blocks bacterial folate metabolism and thus synthesis of new nucleic acids. However, it does it in a completely different manner. Trimethoprim resembles dihydrofolate, and binds to the folate site on DHFR. However, it cannot be reduced by the enzyme and thus acts as a potent inhibitor of the enzyme. All organisms (bacteria and humans included) have DHFR, but there are subtle differences in the structure between the human and bacterial enzymes. Due to this difference, trimethoprim binds ~100,000 times more tightly to the bacterial form of DHFR, and thus has little effect on human folate metabolism. Blocking the reduction of dihydrofolate to tetrahydrofolate has a dramatic effect on nucleic acid production, due to the folic acid cycle shown below. Tetrahydrofolate is converted to the 5,10-methylene form which is then utilized by thymidylate synthase for the production of dTMP. This reaction leads to the oxidation of the cofactor back to the dihydrofolate form, which must then be reduced by DHFR. A complete blockage of folate metabolism can lead to “thymidine-less death” of cells due to dTMP deprivation.
C) Related Drugs
The development of trimethoprim demonstrated that it was possible to make selective inhibitors of DHFR, and that DHFR was a valuable drug target. This has led to the development of the compounds shown below. Methotrexate is an exceptionally potent inhibitor of human DHFR (and bacterial DHFR as well) and is a valuable anticancer agent due to this fact. Pyrimethamine selectively inhibits malarial DHFR and thus is an antimalarial agent. Trimetrexate selectively inhibits \textit{P. carinii} DHFR, and thus blocks the growth of this opportunistic infection in AIDS patients.

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D) Combination Therapy
Trimethoprim is almost always used in combination with sulfamethoxazole as the drug \textbf{cotrimoxazole} (Septra or Bactrim). At the ratio found in cotrimoxazole (5 parts sulfamethoxazole: 1 part trimethoprim), these agents exhibit potent \textbf{synergistic action against susceptible bacteria}. The reason for this synergism is that both of these compounds inhibit the production of tetrahydrofolate in bacteria, but they do so at different steps, and thus allow for a more complete blockade of the folate pathway and thus nucleic acid biosynthesis. This results in a combination that is frequently bacteriocidal, despite the fact that the individual agents are only bacteriostatic. Cotrimoxazole is an important oral and parenteral treatment for gram-negative urinary tract infections, and also finds use in the treatment of \textit{P. carinii} infections and nocardiosis.

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E) Metabolism, Elimination & Toxicity
Trimethoprim, like the sulfonamides, is primarily excreted unmodified in the urine, and this facilitates its function in the treatment of urinary tract infections. Few complications are seen with the use of trimethoprim. Neutropenia can be seen, and the risks of this side effect are decreased by folate deficiency in the patient. Very rarely, dermatological and gastric hypersensitivity are observed.
III. Quinolones

A) Structures

The quinolones are a series of more recently developed, completely synthetic, antibiotics. The first quinolone to be introduced was nalidixic acid in 1965. It was primarily effective for oral treatment of urinary tract infections by susceptible organisms (primarily E. Coli). It exhibited little systemic availability, and resistance to this agent emerged rapidly. Quinolones thus were of limited clinical utility, until the introduction of norfloxacin, the first important fluoroquinolone, in 1986. The introduction of the fluorine atom onto the aromatic ring has two important effects: a) it delays the appearance of resistance to the drug; and b) it broadens the spectrum of action to include many gram negative and gram positive bacterial strains. Norfloxacin is still an agent that is only useful orally, is poorly absorbed, and is primarily useful for urinary tract infections. However, further modification of the fluoroquinolone structure led to ciprofloxacin, the most widely used member of this class. This drug is used both orally and parenterally, exhibits very good absorption, and is useful for a wide variety of gram positive and gram negative bacterial infections. Levofloxacin, a more recently introduced fluoroquinolone, exhibits even better tissue penetration and a wider spectrum of action that ciprofloxacin. Note that numerous other fluoroquinolones are also used clinically.

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\begin{align*}
\text{Nalidixic Acid} & & \text{Norfloxacin} & & \text{Ciprofloxacin} & & \text{Levofloxacin}
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B) Mechanism of Action - *inhibition of DNA Gyrase*

Fluoroquinolones are potently bacteriocidal versus susceptible bacteria due to their ability to inhibit DNA gyrase (bacterial DNA topoisomerase II). In bacterial cells (as in all types of cells) DNA is normally tightly packed for storage. In order to be accessed for transcription or replication, the DNA must be unwound. However, this unwinding process introduces strain (positive supercoiling) into the DNA. This strain is relieved by the action of the enzyme DNA gyrase, which breaks unwinding DNA strands and introduces negative supercoiling into the double helix. Quinolones bind tightly to DNA gyrase, block negative supercoiling, and thus prevent transcription and replication and lead to bacterial cell death. More specifically, a Gyrase-DNA-fluoroquinolone ternary complex is believed to be formed. Our DNA also must undergo negative supercoiling before transcription and replication can occur. However, mammalian topoisomerase II is quite different in structure from bacterial topoisomerase II, and thus quinolones do not bind to our enzyme. This results in a very selective action on bacterial cells.

Topoisomerase IV is another bacterial type II topoisomerase. Topo IV does not supercoil DNA however, it carries out ATP dependant relaxation of DNA. Some fluoroquinolones are known to preferentially inhibit the action of this enzyme in certain gram (+) organisms by stabilizing the topoisomerase-DNA complex and thus halting activity. In fact, different fluoroquinolones typically have different capacities to inhibit DNA gyrase and/or topoisomerase IV. There can also be subtle differences in how each quinolone effects this inhibition.

**Resistance** to fluoroquinolones is primarily mediated by efflux of the agent or by mutations in genes that encode for Gyrase and/or Topo IV.
C) Metabolism and Elimination

The fluoroquinolones are generally well absorbed after oral administration and are widely distributed in body tissues. In particular, the penetration of fluoroquinolones into the prostate makes these agents particularly valuable for the treatment of infections of this gland. Note that Ca, Mg, Al, and Fe ions can interfere with oral absorption of the fluoroquinolones. The primary mode of metabolism varies among the various fluoroquinolones, but typically most of a dose is excreted unmodified in the urine.

D) Toxicity

Toxicity is limited with most fluoroquinolones. CNS side effects are most commonly seen (hallucinations, insomnia, and visual disturbances) with fluoroquinolones. Damage to growing joints was seen in young experimental animals, and for this reason the fluoroquinolones are prescribed with care to children.

IV. Miscellaneous Agents

A) Nitrofurantoin

Also called macrodantin, this oral antibacterial agent has been available since World War II. It is used for prophylaxis or treatment of urinary tract infections. Nitrofurantoin is reduced to give metabolites that alkylate DNA, leading to its damage and the death of the cell. The key to its selective bacteriocidal action is the fact that susceptible bacteria are much better at reducing nitrofurantoin than our cells.

B) Metronidazole

This agent was initially used clinically for the treatment of protozoal infections (e.g., trichomoniasis). However, it is also a potent antibacterial agent against many obligate anaerobic bacteria, such as Clostridium difficile. It has a mechanism of action similar to nitrofurantoin, in that it is reduced to a reactive metabolite that alkylates DNA. This reduction occurs more rapidly in susceptible organisms, such as anaerobic bacteria, than in human cells.