Antibacterial Agents

Differences between bacterial and animal cells

• The bacterial cell has a cell wall, as well as a cell membrane, whereas the animal cell has only a cell membrane. The cell wall is crucial to the bacterial cell's survival. Bacteria have to survive a wide range of environments and osmotic pressures, whereas animal cells do not. If a bacterial cell lacking a cell wall was placed in an aqueous environment containing a low concentration of salts, water would freely enter the cell due to osmotic pressure. This would cause the cell to swell and eventually 'burst'. The cell wall does not stop water flowing into the cell directly, but it does prevent the cell from swelling and so indirectly prevents water entering the cell.

• The bacterial cell does not have a defined nucleus, whereas the animal cell does.

• Animal cells contain a variety of structures called organelles (e.g. mitochondria, etc.), whereas the bacterial cell is relatively simple.

• The biochemistry of a bacterial cell differs significantly from that of an animal cell. For example, bacteria may have to synthesize essential vitamins which animal cells can acquire intact from food. The bacterial cells must have the enzymes to catalyze these reactions. Animal cells do not, since the reactions are not required.

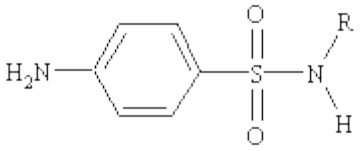
Antibacterial agents which act against cell metabolism(antimetabolites)

1- Sulfonamides (the sulfa drugs).

Sulfanilamide was synthesized in the laboratory and became the first synthetic antibacterial agent active against a wide range of infections. Further developments led to a range of sulfonamides which proved

effective against **Gram-positive organisms**, especially *pneumococci* and *meningococci*.

Despite their undoubted benefits, sulfa drugs have proved ineffective against infections such as *Salmonella*—the organism responsible for typhoid. Other problems have resulted from the way these drugs are metabolized, since toxic products are frequently obtained. This led to the sulfonamides mainly being superseded by penicillin.



Sulfonimides

Structure-activity relationships (SAR)

The synthesis of a large number of sulfonamide analogues led to the following conclusions:

• The *p*-amino group is essential for activity and must be unsubstituted (i.e. R = H). The only exception is when R = acyl (i.e. amides). **The amides themselves are inactive but can be metabolized in the body to regenerate the active compound**. Thus **amides** can be used as sulfonamide prodrugs.

• The aromatic ring and the sulfonamide functional group are both required.

• The aromatic ring must be *para*-substituted only.

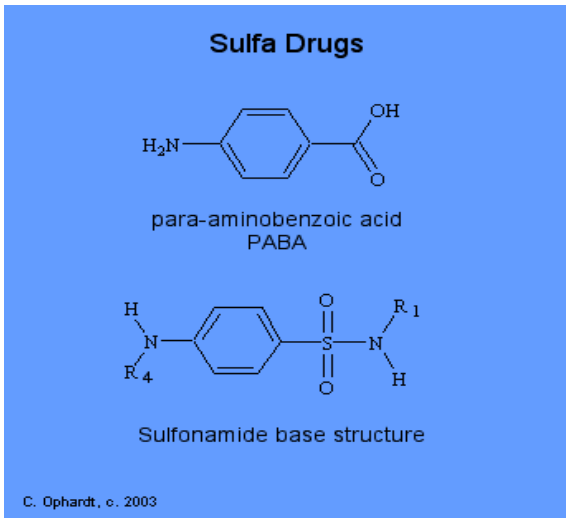
• The sulfonamide nitrogen must be secondary.

• R" is the only possible site that can be varied in sulfonamides.

Sulfanilamide analogues

R" can be varied by incorporating a large range of heterocyclic or aromatic structures which affects the extent to which the drug binds to

plasma protein. This in turn controls the blood levels of the drug such that it can be short acting or long acting. Thus, **a drug which binds strongly to plasma protein will be slowly released into the blood circulation and will be longer lasting.**



Changing the nature of the group R" has also **helped to reduce the toxicity of some sulfonamides**. *The primary amino group of sulfonamides are* ***acetylated*** *in the body and the resulting amides have reduced solubility which can lead to toxic effects*. For example, the metabolite formed from sulfathiazole (an early sulfonamide) is poorly soluble and can prove fatal if it blocks the kidney tubules.

It is interesting to note that **certain nationalities** are more susceptible to this than others. For example, the Japanese and Chinese metabolize sulfathiazole more quickly than the Americans and are therefore more susceptible to its toxic effects.

It was discovered that the solubility problem could be overcome by replacing the thiazole ring in sulfathiazole with a pyrimidine ring to give sulfadiazine. **The reason for the improved solubility lies in the acidity of the sulfonamide NH proton**. In sulfathiazole, this proton is not very acidic (high p*Ka*). Therefore, sulfathiazole and its metabolite are mostly un-ionized at blood pH. Replacing the thiazole ring with a more electron

withdrawing pyrimidine ring increases the acidity of the NH proton by stabilizing the anion which results. Therefore, sulfadiazine and its metabolite are significantly ionized at blood pH. As a consequence, they are more soluble and less toxic.

Sulfadiazine was also found to be more active than **sulfathiazole** and soon replaced it in therapy. To conclude, varying R" can affect the solubility of sulfonamides or the extent to which they bind to plasma protein.

Applications of sulfonamides

Before the appearance of penicillin, the sulfa drugs were the drugs of choice in the treatment of infectious diseases. The sulfa drugs presently have the following applications in medicine:

- treatment of urinary tract infections
- eye lotions
- treatment of infections of mucous membranes
- treatment of gut infections

Sulfonamides have been particularly useful against infections of the intestine and can be targeted specifically to that site by the use of prodrugs. For example, **succinyl sulfathiazole** is a prodrug of sulfathiazole. The succinyl group converts the basic sulfathiazole into an acid which means that the prodrug is ionized in the slightly alkaline conditions of the intestine. As a result, it is not absorbed into the bloodstream and is retained in the intestine. Slow enzymatic hydrolysis of the succinyl group then releases the active sulfathiazole where it is needed.

Substitution on the aniline nitrogen with benzoyl groups has also given useful prodrugs which are poorly absorbed through the gut wall and can be used in the same way.

Mechanism of action

The sulfonamides act as **competitive enzyme inhibitors** and block the biosynthesis of the vitamin folic acid in bacterial cells. They do this by inhibiting the enzyme responsible for linking together the component parts of folic acid. Under normal conditions, folic acid is the precursor for tetrahydrofolate - a compound which is crucial to cell biochemistry since it acts as the carrier for one-carbon units, necessary for many biosynthetic pathways. *If tetrahydrofolate is no longer synthesized, then any biosynthetic pathway requiring one-carbon fragments is disrupted*. The biosynthesis of nucleic acids is particularly disrupted and this leads to the cessation of cell growth and division.

Note that sulfonamides do not actively kill bacterial cells. They do, however, prevent the cells dividing and spreading. This gives the body's own defense systems enough time to gather their resources and wipe out the invader.

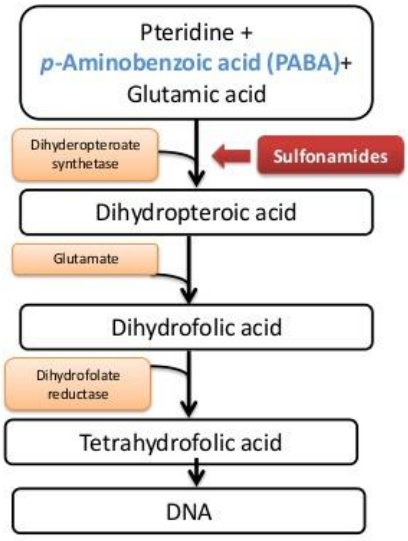
Sulfonamides act as inhibitors by mimicking *p*-aminobenzoic acid (PABA) one of the normal constituents of folic acid. The sulfonamide molecule is similar enough in structure to PABA that the enzyme is fooled into accepting it into its active site. Once it is bound, the sulfonamide prevents PABA from binding. As a result, folic acid is no longer synthesized. Since folic acid is essential to cell growth, the cell will stop dividing.

Sulfonamides are competitive enzyme inhibitors and as such the effect can be reversible. This is demonstrated by certain organisms such as *staphylococci*, *pneumococci*, and *gonococci* which can acquire resistance by synthesizing more PABA. The more PABA there is in the cell, the more effectively it can compete with the sulfonamide inhibitor to reach the enzyme's active site.

Folic acid is clearly necessary for the survival of bacterial cells. *However, folic acid is also vital for the survival of human cells,* **so why do the sulfa drugs not affect human cells as well?** The answer lies in the fact that human cells cannot make folic acid. They lack the necessary enzymes and so there is no enzyme for the sulfonamides to attack. Human cells acquire folic acid as a vitamin from the diet. Folic acid is brought through the cell membrane by a transport protein and this process is totally unaffected by sulfonamides.

Mechanism of action

- Bacteria synthesize their own folic acid (FA) of which *p*-aminobenzoic acid (PABA) is a constituent, and is taken up from the medium.
- Sulfonamides, are structural analogues of PABA, inhibit bacterial folate synthase and formation of folate get inhibited.
- Sulfonamides competitively inhibit the PABA with pteridine residue to form dihydropteroic acid which conjugates with glutamic acid to produce dihydrofolic acid.
- Sulfonamide altered folate an which is metabolically injurious



We could now ask, **'If human cells can acquire folic acid from the diet, why can't bacterial cells infecting the human body do the same?** To sum up, the success of sulfonamides is due to two metabolic differences between mammalian and bacterial cells. *In the first place*, bacteria have a susceptible enzyme which is not present in mammalian cells. *In the second place,* bacteria lack the transport protein which would allow them to acquire folic acid from outside the cell.

Examples of other antimetabolites

2- Trimethoprim

Trimethoprim is a diaminopyrimidine structure which has proved to be a highly selective, orally active, antibacterial, and antimalarial agent. Unlike the sulfonamides, it acts against **dihydrofolate reductase** - the enzyme which carries out the conversion of folic acid to tetrahydrofolate. The overall effect, however, is the same as with sulfonamides - the inhibition of DNA synthesis and cell growth.

Dihydrofolate reductase is present in mammalian cells as well as bacterial cells, so we might wonder why trimethoprim does not affect our own cells? *The answer is that trimethoprim is able to distinguish between the enzymes in either cell. Mutations over millions of years have resulted in a significant difference in structure between the two enzymes such that trimethoprim recognizes and inhibits the bacterial enzyme, but does not recognize the mammalian enzyme.*

Trimethoprim is often given in conjunction with the sulfonamide sulfamethoxazole. The latter inhibits the incorporation of PABA into folic acid, while the former inhibits dihydrofolate reductase. Therefore, two enzymes in the one biosynthetic route are inhibited. This is a very effective method of inhibiting a biosynthetic route and has the advantage that the doses of both drugs can be kept down to safe levels. *To get the same level of inhibition using a single drug, the dose level of that drug would have to be much higher, leading to possible side-effects*. This approach has been described as '**sequential blocking**'.

3- Sulfones

The sulfones are the most important drugs used in the treatment of **leprosy**. It is believed that they inhibit the same bacterial enzyme inhibited by the sulfonamides, i.e. **dihydropteroate synthetase**.